Volume 3 Number 6 December 1957

CLINICAL CHEMISTRY

Journal of the American Association of Clinical Chemists

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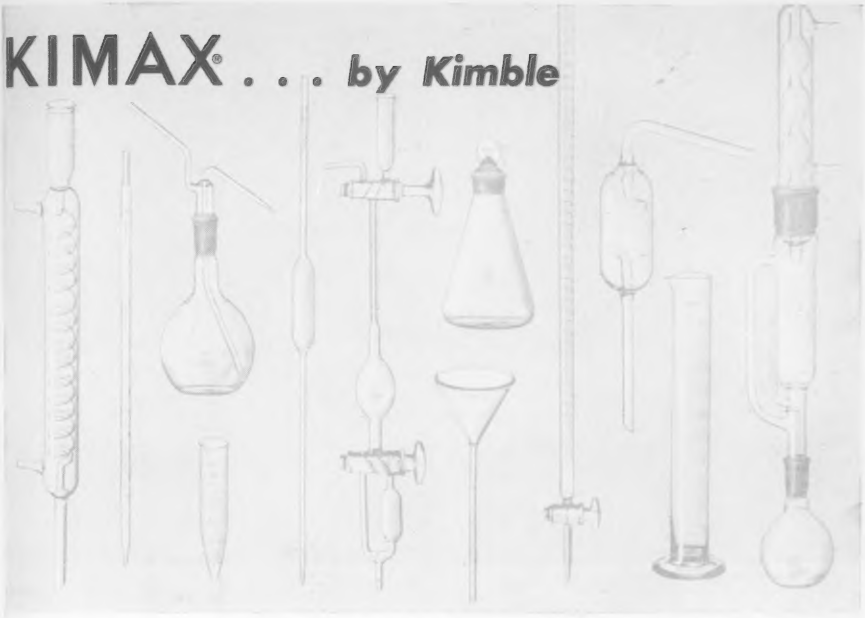

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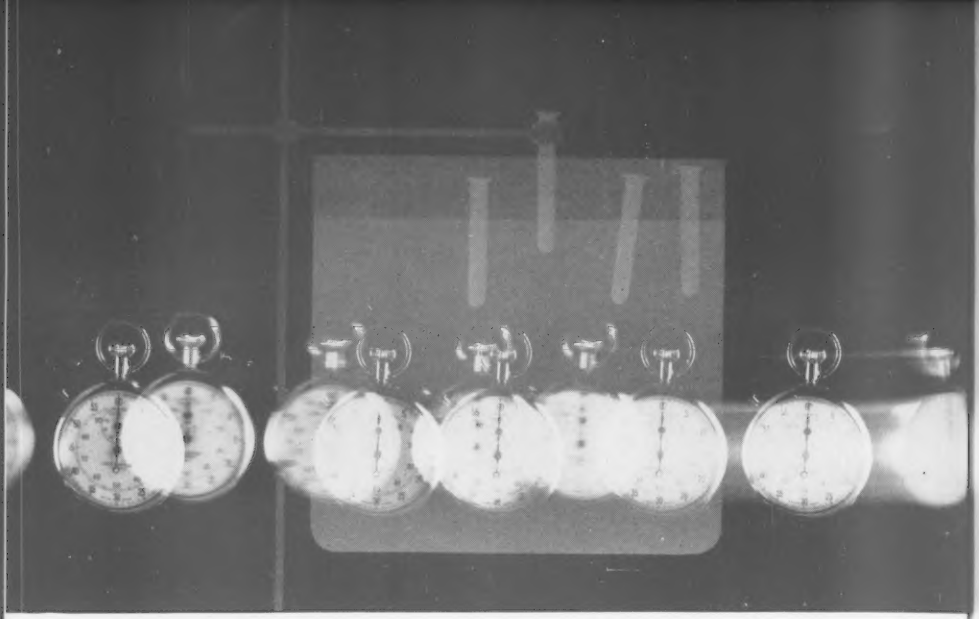

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VOLUME 3 DECEMBER 1957 NUMBER 6

CONTENTS

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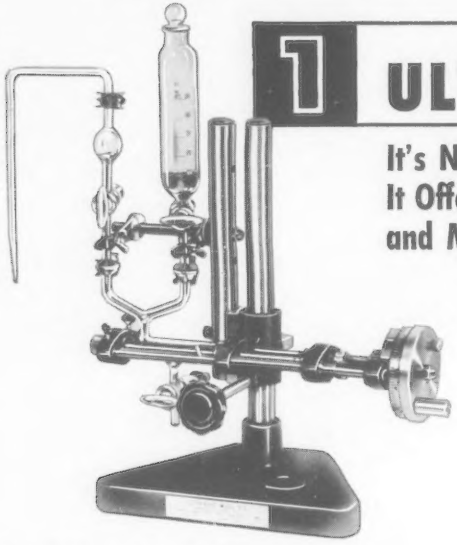

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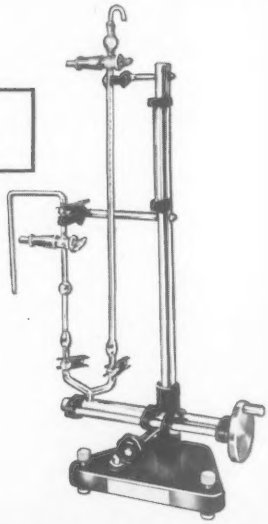

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Published bimonthly at 3110 Elm Ave., Baltimore 11, Md. Second-class mail privileges authorized at Baltimore, Md. Subscription: $8.00 in U. S., possessions, and Pan-American Postal Union; $8.50 in Canada; $9.00 elsewhere. Single copies $1.50. *Printed in U.S.A.*

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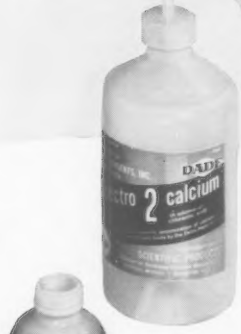

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Subscription Rates: CLINICAL CHEMISTRY is issued bimonthly, one volume per year. The subscription price, payable in advance, is $8.00 in the U. S. A., possessions, and countries in the Pan-American Postal Union, $8.50 per year in Canada, $9.00 per year elsewhere. All subscriptions commence with the first issue of the current volume. Single copies $1.50. Back issues will be supplied at the single copy rate when available.

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Influence of Variations in Body-Fluid Volumes on Sodium Metabolism in Man

Bertil Josephson

THERE IS OVERWHELMING LITERATURE ON how the body handles administered sodium and sodium chloride. Numerous clever and careful experiments on man and animals have been described, and several ingenious theories have been advanced. Nevertheless, we know remarkably little of the mechanism behind the sodium regulation. We know still less of potassium regulation and, accordingly, the potassium literature is less voluminous. This paper will give a short account of recently published investigations on the influence of experimental changes of the volume of body-fluid compartments on the sodium balance in man. Some experiments on this subject carried out in the author's laboratory will also be mentioned. In order to avoid any mistakes about the content of this paper I will declare right away that our experiments are nothing but another stone thrown on the pile. I even doubt that they bring the questions nearer their solution. In this connection some papers (not from the author's laboratory) on the role of hormones in the relationship between fluid volumes and sodium metabolism will be mentioned.

SODIUM BALANCE

The central point in the whole problem is the ability of the healthy organisms to keep a nearly constant store of sodium (and potassium and chloride). This is true not only of warm-blooded animals but also far down in the order of cold-blooded. This may be considered a

From the Clinical Laboratory, St. Erik's Hospital, Stockholm, Sweden.

Presented as part of the Symposium on Electrolytes at the International Congress of Clinical Chemistry, New York, September, 1956.

Editor's note: Our deepest apologies have been extended to Dr. Josephson for not having included his presentation among the symposia which were published as a Supplement to our August publication.

truism, but it is an important truism. If a man or an animal is deprived of sodium in the blood the urine will contain very little sodium. However, the above-mentioned ability is not quite complete and some sodium will always get lost with the urine and the sweat. If this negative balance goes too far, severe symptoms appear. On the other hand, sodium chloride administered by mouth or by injection to an individual healthy in sodium balance will be quantitatively excreted by the kidneys. It may take an hour, it may take days and even weeks, but out it comes.

It must be repeated that this is in the healthy individual. In cases of glomerulonephritis, arterial hypertension, and cardiac failure, the ability to keep the body in salt balance is more or less lost. In these and in some other pathologic conditions an administered surplus of salt may be stored up and increase the total body sodium. This increase may be a heavy burden, as it binds water osmotically, with—or without—recognizable edema as a result.

EXCRETION MECHANISM

There is much work published on the renal mechanism for the excretion of sodium (and chlorides) when the output is considered as the net difference, that is, filtered load from the glomeruli minus tubular back diffusion, back resorption, and returning of sodium to the blood by ion exchange in the tubules. Most of this work is now 5 years old or more and has been carefully reviewed by Homer Smith (1951). Under normal circumstances some 99 per cent of the filtered sodium is reabsorbed in the tubules and 1 per cent appears as the sodium output. Thus, very small changes in the percentage of reabsorption will result in big changes of the net sodium output. This being so, the relative constancy of the sodium excretion is remarkable. This constancy depends on the fact that the tubular reabsorption is such a clever companion to the glomerular filtration rate (GFR) that even if the latter is changed—by experimental procedures or pathologic conditions—the salt excretion does not vary appreciably unless the filtration reaches an extremely low value (and provided the salt intake is constant). If, on the other hand, the salt intake is changed, the amount of sodium filtered in the glomeruli does not change appreciably, but the sodium content of the body is kept fairly constant by changes in tubular reabsorption. In man these changes may be rather slow. In recently published experiments Wesson and Anslow (1955) succeeded in varying the GFR in dogs within wide limits by saline

infusion (increase) or profuse bleeding (decrease). The relationship "GFR over chloride excretion" was linear, provided the plasma chloride concentration was constant. This means that the chloride (and consequently the sodium) reabsorption followed the filtered load very closely. In normal man the tubular reabsorption is still more clever, as its main employer is the need of sodium conservation or sodium excretion, not the filtered sodium.

On the other hand, if the active part of the tubular reabsorption is abolished, the GFR together with the plasma concentration (the filtered load) will become the sole determinant of the excretion. This may be the case after adrenalectomy. Garrod, Davies, and Cahill (1955) found very good correlation between GFR and sodium excretion in adrenalectomized dogs. This correlation disappeared when desoxycorticosterone was given.

Normally some 500 Gm. sodium are filtered per 24 hours in the healthy man. It is generally considered that the main part of this filtered sodium load (80–85 per cent according to Homer Smith) returns from the tubular lumen (probably the loop of Henle) to the extracellular fluid by inevitable diffusion, and that the real regulation occurs from the remaining 15 per cent (Wilson, Anslow, and Smith, 1948). What is left then appears as sodium excretion. If this assumption is correct, the riddle of how sodium balance is maintained is thus concerned with the question of how this 15 per cent is handled and why an appropriate part of it is reabsorbed.

We know very little of the mechanism for tubular absorption. As the sodium balance is maintained, even in diabetes insipidus, and as the urine can be very hypotonic, the reabsorption can take place against a concentration gradient. Thus reabsorption must be a process requiring energy. But usually the urine is hypertonic, which either means that water has been reabsorbed against a concentration gradient or that sodium has been secreted from the tubules. The latter sounds strange but the possibility of a sodium excretion cannot be excluded and an exchange of sodium ions against potassium is not improbable (Black and Mills, 1954). Ek and Josephson (1953) found that if 670 ml. ordinary beer is consumed, sodium excretion increases and potassium decreases by about equal numbers of ions. That potassium can be excreted by the tubular epithelium has been shown by Berliner and his group.

How an excess or a deficit or sodium "tells" the tubular epithelium of its existence is practically unknown and available data are very

confusing. It must be pointed out that great caution is necessary when conclusions about human kidney physiology are to be drawn from animal experiments, as there are great species differences. Intravenously given physiologic saline is rapidly excreted by the dog but very slowly by man. But Ladd (1951) found that he could "make a man into a dog" by letting him drink 2 or 3 liters of plain water. If physiologic saline was given 8–13 hours after the drinking water (which was already quantitatively lost as urine) the saline was excreted as rapidly as by the dog.

The time it takes to excrete a given surplus of salt is nearly independent of how the salt is given. Recently Papper *et al.* (1956) found that the rate with which sodium was excreted after an intravenous injection of 340 mEq. sodium chloride to a healthy man was the same whether the salt was given as a 0.9% or as a 5% solution. The weak solution increased the serum sodium concentration very little, but the chloride space was considerably augmented. The concentrated solution gave a high serum sodium but gave only little change of the chloride space. Their conclusion was that an augmentation of the extracellular fluid volume and a rise of the serum sodium level give about the same increase of the sodium excretion. O'Connor (1955) made corresponding observations on the dog.

It has been maintained that an enlargement of the extracellular fluid volume can cause an increased sodium output in a subject given a surplus of sodium. There is no doubt that such an enlargement can cause an increased water diuresis. A rapid increase of the urine volume can easily give the impression of a transient sodium increase, because the large volume of diluted urine suddenly gushing through and from the kidneys will sweep with it concentrated, sodium-rich urine standing in the tubules and the urinary tract (the dead space).

HORMONE EFFECT

Several experiments on the influence of plasma volume and extracellular fluid volume on sodium excretion have been published. In most of these papers, however, it is not maintained that the fluid spaces per se influence sodium output but that changes in the spaces exert an influence on endocrine organs, the hormones of which govern the tubular adjustment of the urinary sodium.

PITRESSIN

For many years pitressin has been in the center of this discussion. It has to be mentioned in this connection, as its main effect is a regula-

tion of the water balance. The hormone has been credited with both accelerating and restraining properties on sodium excretion, but its effect on the salt balance has often been mixed up with the susceptibility of its production to the osmolarity of the extracellular fluid. Most of this literature has been critically reviewed by Smith. It is well known that patients with diabetes insipidus can take care of their sodium balance if they get enough water, and corresponding observations have been made in hypophysectomized rats. Recently Jacobson and Kellogg (1956) found that pitressin given to hydrated rats gives a pronounced increase of chloride excretion, concomitantly with the antidiuretic effect.

Leaf and coworkers (1953) studied the influence of hormones on the sodium excretion. They found that single, large injections of pitressin to healthy human subjects did not change their sodium output appreciably in spite of the fact that it increased the body water volume by depressing the diuresis. But if the hormone was given in small repeated doses for several days, the sodium excretion rose considerably on the third day of injection, provided the subject was well hydrated. It was without effect on the sodium balance in the dehydrated man. But its antidiuretic effect was evident during the whole period of injections. They did not think that pitressin has any direct effect on how the sodium is handled, but that its natriuretic effect is due to a secondary effect in some other organ, probably the adrenals. Anslow and Wesson (1955) made similar observations on the dog. They consider pitressin to have a depressing effect on the tubular reabsorption of sodium. Their explanation of the fact that pitressin does not work in dehydrated animals is that during hemoconcentration the amount of circulating endogenous pitressin is so high that the injected amount does not make much difference to the kidneys.

ALDOSTERONE

The whole question of hormonal influences on salt balance has to be reconsidered since it was found that corticosteroids from the adrenal cortex have a profound influence on the salt metabolism. In an interesting review John P. Merrill described the position of research in this field in 1953. Since then, however, many papers on this subject have appeared.

Of great importance for our knowledge of salt balance is the discovery of aldosterone and the fact that this cortical steroid seems to be the most potent hormone for sodium conservation. Due to lack of

material, this hormone has been little studied so far, but it seems probable that it will open entirely new aspects on the problem. Its formula was published in 1953 by Simpson *et al.* The hormone has been found both in blood, urine, tissues, and body fluids (Neher and Wettstein, 1955), and the urinary output is found to be augmented in cases of cardiac insufficiency and nephrosis (Bartter, 1953) and in arterial hypertension (Genest *et al.*, 1956). Recently Luetscher, Dowdy, and Harvey (1955) were able to prepare it in pure form from the urine of a nephrotic patient. Now it has been found by two different groups, independently and simultaneously, that hydration of normal human subjects by water and pitressin results in a decreased aldosterone excretion, and that dehydration gives an increase of this hormone in the urine (Bartter *et al.*, 1956; Muller *et al.*, 1956). Hetzel *et al.* (1956) found the sodium-retaining effect of aldosterone to be 30 to 40 times stronger than that of desoxycorticosterone acetate.

BLOOD PRESSURE EFFECT

It is well known that changes in the hemodynamic equilibrium can be of influence on the salt metabolism. This question too was summed up by Merrill in his above-mentioned review of 1953. As changes in the blood volume may influence the blood pressure, the effect of this pressure on sodium excretion may be mentioned. In animal experiments Bradley *et al.* (1955) increased the blood pressure in a renal vein. The result was a considerable decrease of sodium output from this kidney. At the same time the renal blood flow was greatly diminished. It has also been found that the sodium (and potassium) output decrease if the cardiac output and the renal blood flow decrease due to an injection of norepinephrine (Nickel *et al.* 1954).

Also the intracranial blood pressure seems to be of influence on the salt excretion. Strauss *et al.* (1952) found that this excretion was much greater when a salt infusion was given to a recumbent person than to one sitting. However, the same group found the sodium excretion to be higher in a hydrated subject standing than one sitting in an erect position. O'Connor (1955) gave intravenous infusions of salt solution to trained dogs. Occlusion of the carotid arteries immediately resulted in an increased excretion of water and salt and upon release of the occlusion the natriuresis and diuresis immediately returned to the preocclusion values. Recently Surtshin and White (1956) found the sodium excretion of a well-hydrated man to be higher in a recumbent position than when standing erect (the same

was found for GFR and diuresis). On the other hand, Cathcart and Williams (1955) found no difference in sodium excretion between erect and recumbent positions in a normal man without a salt surplus.

Earlier it has been mentioned that an expansion of the extracellular fluid volume is considered to speed up the excretion of an administered surplus of sodium. Rosenbaum and Strauss and their groups have published extensive studies on this question. If a concentrated albumin solution was given intravenously to a healthy man the bulk of extra water stayed in the vascular system and no increase of the sodium output occurred; in fact a decrease was usually observed (Strauss *et al.*, 1952). In later papers they described experiments showing that in healthy man the water is excreted more rapidly than the salt if physiologic saline and hypotonic salt solutions are given (Birchard, Rosenbaum, and Strauss, 1953). Earlier Petersdorf and Welt (1953) also had found a decreased sodium excretion when albumin was given intravenously to persons with a water surplus of 1 liter (by injection of concentrated albumin).

EFFECT OF EXPANSION OF ECF AND BLOOD VOLUME IN NORMAL MAN

In order to study the effect of an expansion of the extracellular fluid without administration of sodium, Ek (1955) in our laboratory infused large amounts of *iso-oncotic glucose solution in healthy persons*. The experiments were described in a dissertation. About 25 ml. glucose solution was given per minute and simultaneously the clearance of PAH, inulin, and creatinine, and the excretion of sodium, potassium, and chlorides were followed. The diuresis increased rapidly during the infusion, but not until after about 1 hour had it reached a level similar to the infusion rate. Then about 1 liter of water was retained in the body. The sodium excretion, however, was practically constant or increased very little. One of Ek's experiments is illustrated in Fig. 1. One year earlier Migone, Ambrosoli, and Scarpioni (1954) had published similar experiments on healthy subjects. In contrast to Ek they had found an increase of the sodium excretion with increasing diuresis. In Ek's experiments on normal subjects the inulin clearance increased a little and serum sodium decreased a little but the net result was a slight increase of the calculated filtered sodium load. Thus the tubular reabsorption must have increased accordingly.

An expansion of the intravascular fluid volume without changing

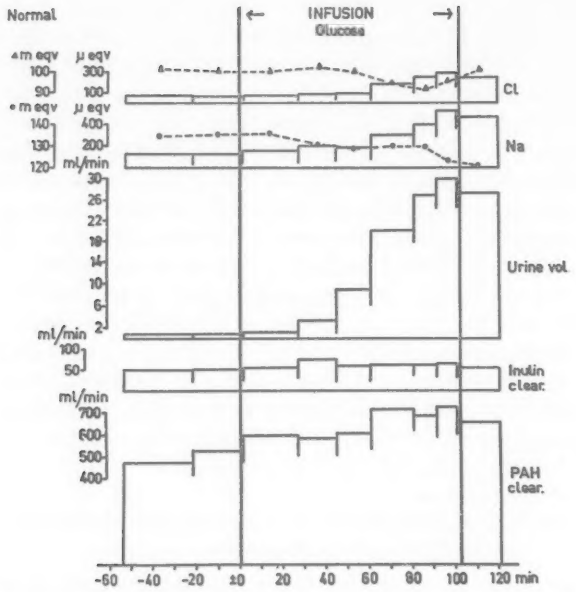

Fig. 1. The effect of intravenous infusion (between the vertical lines) of 28 ml./min. of 6% glucose solution to normal man (from Ek, 1955). The staples represent urinary excretion resp. clearances, the dotted lines plasma concentrations.

the extravascular, extracellular fluid volume has been achieved by infusion of isotonic albumin solution, human plasma, or isotonic dextran solution. Some of the albumin experiments have been mentioned above. Welt and Orloff (1951) and Strauss *et al.* (1952) found iso-oncotic "albumin expansion" to be without appreciable effect on the sodium output.

In our laboratory a group consisting of Bucht, Ek, Werkö, Eliasch, Schnabel, and myself has studied the effect of the intravenous infusion of large amounts of *iso-oncotic dextran infusions in normal man.* The intention was to study the effect of a selective expansion of the plasma volume on the excretion of electrolytes and on renal hemodynamics. Unfortunately, a solution of nothing but dextran cannot be given in large amounts without causing hemolysis. Therefore the commercial dextran solutions contain an isotonic concentration of either glucose or sodium chloride. We have tried both. Between 25

Table 1. INTRAVENOUS INFUSION OF DEXTRAN SOLUTION CONTAINING 10% GLUCOSE INTO 9 HEALTHY HUMAN SUBJECTS

Time*	Diuresis	Clearance (ml./min.)		Excretion (μEq./min.)		Serum Na (mEq./l.)
		Creatinine	PAH	Na	Cl	
Basal	2.5	114	572	306	238	132.9
0–25	2.9	107	661	285	199	131.3
25–50	5.0	111	749	212	143	126.9

*Infusion rate 25–30 ml./min. Basal mean values obtained before the start of infusion at time 0.

and 30 ml. were given per minute. As the infusion was kept going during 1 hour or more, about 1600 to 1800 ml. were given altogether.

When dextran-glucose was given to healthy subjects the sodium output followed a rather steep downhill slope (Table 1). The decrease started even during the first period of infusion when no changes in the serum sodium could be observed. It continued to decrease even after the infusion was finished. At the same time the serum sodium went down too. Due to the dextran in the samples inulin clearance was not studied, but the endogenous creatinine clearance did not change significantly. As this clearance cannot be considered as a reliable measure of the GFR, the tubular sodium load could not be calculated; but because serum sodium and serum chloride usually decreased, the load must have been lowered too. Later during the infusion both sodium and chloride decreased considerably and then the filtered load decreased more than the sodium excretion did. During the later periods of infusion (25–50 minutes after the start) the sodium output was only 50–60 per cent of the basal value (Table 2). The chloride values closely followed those for sodium. The diuresis rose moderately but during the infusion it never reached higher values than about two to three times the basal value. It continued to rise after the infusion was finished. One experiment is illustrated in Fig. 2.

In the experiments with dextran containing sodium chloride (Table 3) the sodium balance gets disturbed by the infused salt (in contrast to the dextran-glucose experiment). Nevertheless, there was often a decrease in the output of sodium and chloride during the first period after commencement of the infusion. Subsequently the urinary sodium excretion did not increase until after about 1 hour when the infusion was already finished (Table 4). In these experiments the serum levels of sodium and chloride changed only transiently, but it

Table 2. Ratio Between Found Mean Values for Different Periods Before, During, and Immediately After Infusion of Dextran-Glucose, and Significance of These Ratios (Same material as Table 1 but figures calculated from real periods, usually about 15 min., not from interpolated periods)

	Diuresis		*PAH clearance*		*Creatinine Cl*		*Na excretion*	
	M	*p*	*M*	*p*	*M*	*p*	*M*	*p*
$\frac{\text{per } f}{B}$	0.93[a]	0.3	–	–	0.89	0.1	0.83[a]	0.025
$\frac{\text{per } l}{B}$	2.67	0.025	1.45	0.05	1.00	1	0.57	0.05
$\frac{\text{per } a}{B}$	3.72	0.05	1.46	0.01	1.09	0.1	0.58	0.01
$\frac{\text{per } a}{\text{per } l}$	1.57	0.025	1.00	1	1.13	0.1	1.02	1

B = basal, per f = first period during infusion, per l = last period during infusion, per a = first period after end of infusion, M = arithmetical mean, p = random probability.

[a] In calculation of this mean one figure was omitted. This was from an experiment which gave an untypical result in nearly every respect.

is probable that the sodium load was decreased nevertheless, as the creatinine clearance sometimes went down by 15 to 20 per cent. As soon as the infusion was over, and simultaneously with the increase in sodium output, the creatinine clearance rose (Fig. 3). But the variations are high and the figures are not significant. The Hb and protein figures show that the plasma volume increased up to 20 per cent in the dextran experiments.

Corresponding control experiments with infusion of an isotonic solution of sodium chloride alone are now in progress.

In most of our experiments the pressure in a pulmonary artery was determined. During the infusion it always increased by 50–100 per cent. In a few experiments the pressure in a renal vein was determined. This pressure too was considerably increased.

The glucose experiments (without dextran) provide further evidence that an acute slight increase of the extracellular fluid volume is without effect on the salt excretion in normal man. An expansion of the plasma alone, on the other hand (as in the dextran experiments), seems to depress the sodium output. However, we cannot exclude the possibility that there is some special and unknown effect of dextran on the sodium balance. Expansion of the blood volume by dextran infusion in the dog is followed by increase of the diuresis, the GFR, and the renal blood flow, especially if the infusion is given slowly (Young,

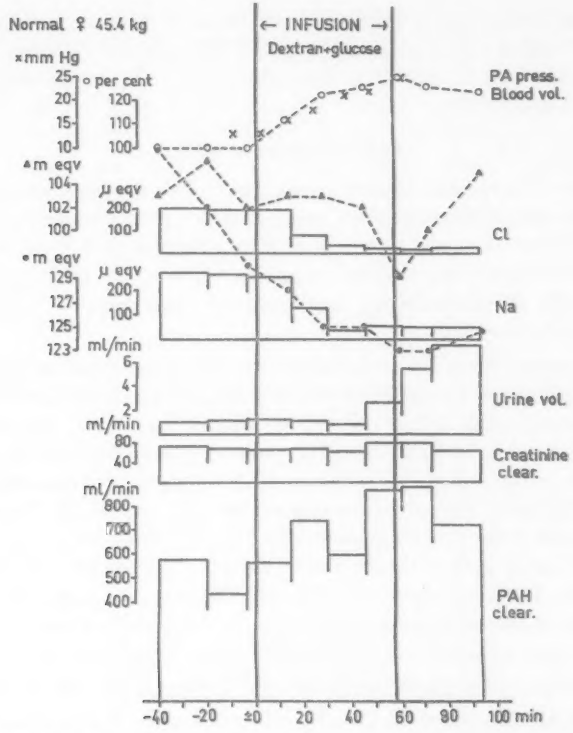

Fig. 2. The effect of intravenous infusion of 25 ml./min. of a solution containing 10% dextran and 6% glucose to normal man. o = relative blood volume in per cent of basal value. x = pressure in pulmonary artery (PA) in mm. Hg. Legend otherwise as in Fig. 1.

Table 3. INTRAVENOUS INFUSION OF DEXTRAN SOLUTION CONTAINING 0.9% NaCl INTO 7 HEALTHY HUMAN SUBJECTS

Time[a]	*Diuresis*	*Clearance (ml./min.)*		*Excretion (μEq./min.)*		*Serum Na (mEq./l.)*
		Creatinine	*PAH*		*Cl*	
Basal	2.2	101	572	432	307	135.6
0–25	2.3	85	576	417	300	135.3
25–50	2.3	82	633	421	311	132.7

[a]Same as Table 1.

Pearce, and Stevenson, 1956). In our experiments a very small part of the infused dextran went to the urine. The concentration of dextran in the urine was so low that its influence on the osmolarity of the urine was negligible.

IN CIRCULATION FAILURE

In cases of *arterial hypertension, and of heart failure,* the basal conditions are different from the normal. These patients seem to have a hidden or secret store of sodium chloride and they may have this store without any clinical signs of edema. In these patients a shower-bath of water given intravenously can support the sodium excretion enormously.

Many papers describe experiments on the influence of water supply on salt excretion in patients of this kind. Crutchfield and Wood (1948) found that water given by mouth increases diuresis and sodium excretion in patients with poor diuresis but not in patients with good urinary flow. It seems probable that the former but not the latter ones had a sodium store caused by salt retention. Green *et al.* (1954) studied the sodium output after intravenous infusion of 5 Gm. sodium chloride into patients with arterial hypertension. In the early state of the disease, when the kidney function was good, the sodium given was excreted more rapidly than in healthy subjects, while the excretion was slower in the advanced state. The retention of water and of sodium in patients with several kinds of cardiac failure was studied by Hanenson *et al.* (1956). They consider that proportionally more water is retained than the corresponding amount of salt. By infusion of a 5% glucose solution the output of urine was increased. The sodium excretion varied with the volume of the extracellular fluid. It did not change when the diuresis was depressed by injection of pitressin.

In the dissertation, mentioned above, Ek (1955) described experiments in which about 25 ml. per minute of an *iso-oncotic glucose solution* were given not only to healthy subjects but also *to patients with arterial hypertension.* In these patients the result was dramatically different from the normal response. In contrast to the result in the normal subjects the sodium excretion started rising from the start of the infusion, and it continued to rise until it had—after about 1 hour —reached a value of more than three times the basal one. In some of Ek's experiments the final sodium output was 10 times the original.

Table 4. The Ratio Between Found Mean Values for Different Periods Before, During, and Immediately After Infusion of Dextran-NaCl (Same material as Table 3)

	Diuresis		*PAH clearance*		*Creatinine Cl*		*Na excretion*	
	M	*p*	*M*	*p*	*M*	*p*	*M*	*p*
per f / B	0.97	0.9	--	--	0.87	0.05	0.90	0.1
per l / B	1.26	1	1.23	0.1	0.88	0.2	1.03	1
per a / B	1.49	0.1	1.49	0.005	1.05	0.2	1.23	0.1
per a / per l	1.31	0.025	1.33	0.1	1.30	0.1	1.28	0.05

B = basal, per f = first period during infusion, per l = last period during infusion, per a = first period after end of infusion, m = arithmetical mean, p = random probability.

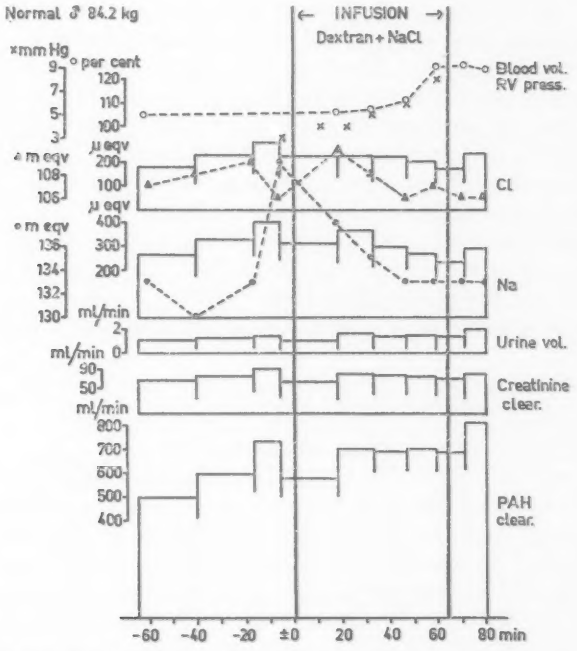

Fig. 3. The effect of intravenous infusion of 25 ml./min. of a solution containing 10% dextran and 0.9% NaCl to normal man. x = pressure in the left renal vein (RV). Legend otherwise as in Fig. 2.

And in contrast to Crutchfield and Wood (1948), Ek found this phenomenon even in considerably severe cases.

The diuresis also increased, as it did in the normal. The difference was that in the normal the diuresis and the sodium output were quite independent of each other, while there was a nice proportionality between water and sodium output in the hypertensives—there was a "companionship" between water and salt excretion (Table 5). The correlation between diuresis and sodium excretion in hypertension has earlier been pointed out by Weston *et al.* (1950). In Ek's patients the PAH and inulin clearances also increased much more than in the normals, but this increase was not as obvious as for sodium.

Subsequently our group has found similar results in patients with mitral stenosis. It may be pointed out that unmistakeable results were achieved even in cases with relatively mild symptoms and without any signs of edema.

After an infusion time of about 100 minutes the water retention could be calculated to about 750 ml. in the normal and to about 1100 ml. in the hypertensive. However, these figures do not always correspond to the real water retention, since in some of the subjects slight vomiting or diarrhea occurred. The hemoglobin concentration of the blood and the protein concentration of the plasma did not decrease by more than about 2 per cent, indicating that the retained water was distributed over the whole body water volume.

The administration of the glucose in the iso-oncotic solution may have been of some influence on the potassium balance, but it is not probable that the glucose itself had anything to do with the sodium and chloride metabolism. Brod, Fejfarová, and Chytil (1954) found

Table 5. Intravenous Infusion of Isotonic Solution into 6 Healthy Human Subjects and 13 Patients with Arterial Hypertension (Figures from Ek, 1955.)

	Time[a]	*Diuresis*	*Clearance (ml./min.)*		*Na excretion (μEq./min.)*	*Serum Na (mEq./l.)*
			Inulin	*PAH*		
Normal	Basal	3.2	144	791	424	132
	0–50	10.4	182	906	435	125
	50–100	23.6	169	860	425	124
Hypertensive	Basal	2.8	73	331	253	138
	0–50	6.2	94	478	457	134
	50–100	18.1	100	488	761	130

[a]Same as Table 1.

that a concentrated glucose solution given intravenously can give an augmentation of the renal blood flow when this is decreased due to cardiac failure. In Ek's experiments the increase in blood sugar was slight and glucosuria was absent or very low. In some experiments insulin was added to the infusion fluid with the result that the blood sugar was increased only during the first infusion period and no glucose appeared in the urine. This did not change the sodium results.

Thus it seems as if the only "direct" effect of the infusion of isooncotic glucose is an expansion of the total body water volume, including the extracellular fluid and a corresponding dilution of its solutes. It cannot be answered which of these two effects is responsible for the sodium effect in hypertension and heart failures, but it must be pointed out that the increase starts immediately at the onset of the infusion, and previous to signs of expansion. So did the increase of the GFR.

The considerable increase of the filtered sodium load from the glomeruli corresponds to many times the increases in sodium output. Thus, the tubular reabsorption was increased too, but not in proportion to the filtration rate. In fact, the infused patients behave similarly to the above-mentioned adrenalectomized dogs of Garrod, Davies, and Cahill (1955). That there is a relationship between GFR and sodium excretion in arterial hypertension has been observed earlier (Weston and coworkers). It ought to be mentioned in this connection that Crutchfield and Wood (1948) found a correlation between natriuresis and diuresis in patients with heart failure and that Green *et al.* (1954) found that the sodium excretion was faster in cases of hypertension than in normals when large amounts of physiologic saline were given.

In our laboratory the *infusion of dextran* has been used also *on a number of patients with hypertension and cardiac failure.* Either glucose or sodium chloride was used to make the solution isotonic. At the time of writing the obtained values have not yet been treated statistically. However, the results show that both in cases of cardiac failure and in hypertension the sodium and chloride excretion decreased very much during the infusion of about 25 ml./min. of a dextran solution containing 10% glucose (Fig. 4). In this respect the patients behaved similarly to the normals. In most of these experiments the creatinine clearance did not go down. Usually the sodium output rose again during the first or second period after the end of the infusion.

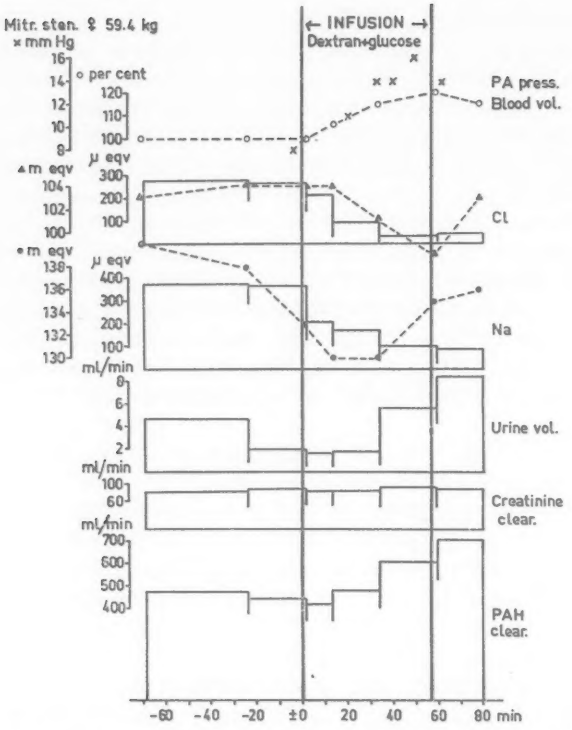

Fig. 4. The effect of intravenous infusion of a dextran-glucose solution (as in Fig. 2) in a patient with mitral stenosis. Legend as in Fig. 2.

In these cases it was not considered advisable to continue the infusion for more than 20–40 minutes. During the first 20–30 minutes of the infusion the diuresis did not change much, but stayed on a level of 1–2 ml./min. At the end of the infusion it usually started to rise. It rose still more after the infusion ended and reached a volume of 5–15 ml./min. The net result of the water balance was an augmentation of the plasma volume of about 400–800 ml. at the end of the infusion. In these experiments vomiting or diarrhea did not occur. This fluid retention corresponded nicely to the decrease in hemoglobin and plasma protein, indicating that all the retained fluid stayed in the blood vessels, increasing the plasma volume.

When dextran containing sodium chloride was given to patients of this kind (instead of dextran-glucose as mentioned above) the sodium output was decreased or stayed more or less constant, but the results were widely varying. Usually the output of urine did not change either and the increase toward the end of the experiments which was found in the dextran-glucose experiments was not observed here (Fig. 5).

COMPARISON: NORMAL-PATHOLOGIC REACTION

It must be admitted that it is not possible to draw any definite conclusions on the central mechanism for salt excretion from our results and that the arrangements of the experiments make several different

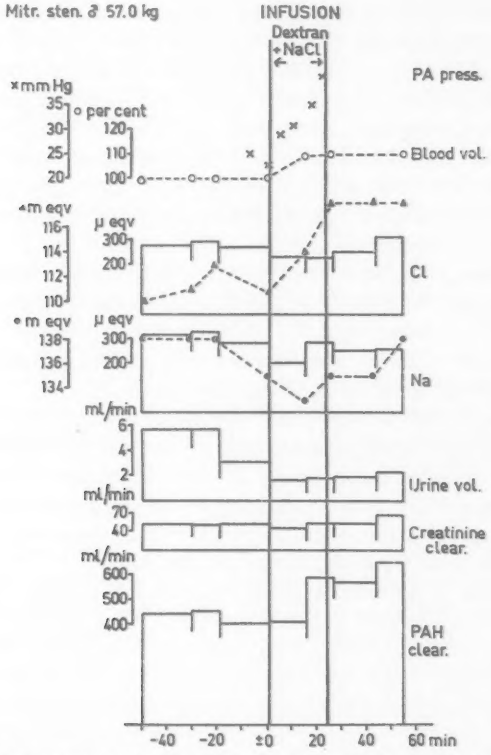

Fig. 5. The effect of intravenous infusion of a dextran-NaCl solution (as in Fig. 3) in a patient with mitral stenosis. Legend as in Fig. 2.

interpretations plausible. It would appear that a rapid iso-oncotic intravenous infusion, giving not more than a slight increase of the body water volume, has no influence on the salt excretion in healthy man but gives rise to a great and rapid increase of this excretion in hypertensive patients and in cases of cardiac failure. On the other hand, this increase may be secondary to the great increase in glomerular filtration rate and tubular sodium load appearing in the hypertensives during the infusion. The above-mentioned observation, made by several investigators, that pitressin given to the well-hydrated man can give rise to an increased sodium excretion, may be interpreted as indicating that a more heavy hydration can increase the sodium output even in the normal man.

On the other hand, an iso-oncotic expansion of the plasma volume (by iso-oncotic dextran) without expansion of the extravascular extracellular fluid seems to cause a decrease of the salt excretion both in healthy subjects and in patients with the above-mentioned diseases. But again, here we cannot completely exclude the possibility that the dextran per se has some hitherto unobserved effect on the salt metabolism. Earlier Petersdorf and Welt (1953) found a reduced excretion of sodium and chloride when the plasma volume was expanded by infusion of 25% albumin solution. In their experiments the extravascular fluid volume must have been correspondingly decreased. It ought to be pointed out that in all our experiments discussed here the subjects have been recumbent. As mentioned above it has been maintained that there may be considerable differences in sodium excretion due to the body position of the subject.

It seems as if there were many quite-different physiologic incitements for changes in the salt excretion. But most of them have little effect, or are without importance, in the salt balance of normal man. A condition for their effect seems to be an administered surplus of extra salt. An exception is the decrease of salt excretion during plasma expansion by dextran or albumin solution.

I believe that in arterial hypertension and in cardiac failure there is a pool of stored-up salt in the body fluids. The excretion of this can be increased by several means without administration of diuretic or natriuretic drugs. For instance, one of the means promoting the excretion is expansion of the extracellular, extravascular fluid volume by the infusion of water or by drinking beer. On the other hand, an expansion of the plasma volume (by dextran) seems to support sodium retention. Our results will soon be published in *Clinical Sci-*

ence and in *The Scandinavian Journal of Clinical and Laboratory Investigation.*

As mentioned earlier the well-known fact that the salt balance is subject to hormonal influences makes it tempting to explain experimental results of the type described here by depression or increase of hormonal discharges. As our knowledge of the action of the hormones known to be of influence on the salt balance is still very limited, and as we have no methods for their quantitative estimation in sufficiently small amounts of blood and urine, theoretical explanations of their influence on the salt balance had better wait until there is further direct evidence. In our present position conclusions on the influence of hormones on the salt excretion should be restricted to experiments in which the respective hormones themselves are used or abolished or determined with reliable methods. However, it has been definitely shown that the production of aldosterone is regulated by changes in the extracellular fluid volume, not by changes in the total sodium supply (Bartter, 1956).

Nervous reflexes may also be of influence, and we know that they are of great importance for the renal blood flow. But according to O'Connor (1955) even the denervated kidney is subject to influences on sodium excretion of the same kind as in the intact animal.

There are many other ways to influence the salt and water metabolism experimentally, such as exercise, drinking large quantities of water, beer, or alcohol, the injection of nicotine, etc. There are also several other pathologic conditions of importance for the retention and excretion of water and sodium, such as liver cirrhosis, nephrosis, etc., which have not been mentioned in this short survey. A discussion of these experiments and conditions would necessarily be far too extensive.

SUMMARY

A review has been given of recent papers on the influence of acute changes in extracellular, extravascular fluid volume and in plasma volume on the salt metabolism. The influence of intravascular pressure, pitressin, adrenal steroids, etc., has been mentioned. Some work from the author's laboratory has been reported. It was found in this laboratory that a large intravenous infusion—25 ml./min. during 1–2 hours—of iso-oncotic glucose has no influence on sodium excretion in normal man, but that it gives rise to a very high increase of the sodium output in cases of arterial hypertension. Later on it was

found that patients with cardiac failure behave as the hypertensive do in this respect. On the other hand, an intravenous infusion of a corresponding amount of iso-oncotic dextran solution gives a considerable decrease of sodium excretion in healthy subjects as well as in patients with the above-mentioned diseases. The correlation between these results and the changes of the kidney function and the renal hemodynamic have been discussed.

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Effects of Delayed Examination on the Results of Certain Hepatic Tests

Virginia L. Yonan and John G. Reinhold

A NEED FOR INFORMATION concerning the validity of hepatic test results obtained from refrigerated blood or serum arose in connection with a blood donor screening program. As a part of this program, hepatic tests were applied to every donor reporting to the donation station for the purpose of excluding those carriers of viral hepatitis or others suffering from subclinical illnesses associated with disturbances in metabolism that cause abnormal response to such tests. Ordinarily these tests are applied to freshly collected blood serum; however, this was not feasible under the circumstances. Therefore, experiments were planned to show (a) whether refrigeration of serum caused changes that significantly altered test results, and (b) whether leaving serum in contact with cells overnight changed its behavior.

Friedman reported that the zinc sulfate turbidities were lowered after refrigeration of sera (12). However, we have not found other reports on the effect of storage of serum or blood on hepatic tests.

METHODS

The tests evaluated included thymol turbidity (1, 8), using reagents buffered at *p*H 7.80 and *p*H 7.55, cephalin-cholesterol flocculation (2, 3), zinc sulfate turbidity (4), phenol turbidity (5), and prompt-react-

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This study was supported in part by the Office of the Surgeon General, Department of the Army.

Received for publication April 17, 1957.

ing (1 minute) and total serum bilirubin (6, 7). All measurements were done in duplicate.

Sera and reagents used for these procedures were maintained at 25° in all experiments subsequent to the first series (9).

RESULTS

THYMOL AND ZINC TURBIDITIES

Sera separated within 2 hours from the bloods of a group of blood donors selected at random (Series 1), and from hospitalized patients (Series 2), were tested both before and after overnight refrigeration. Some of the hospitalized patients had disturbances of liver function. Thymol and zinc turbidities tended to be lower following refrigeration of sera (Table 1, Series 1 and 2). Differences between the means were significant. Calculation of t (13) for the thymol data of Series 2 gave 6.49 ($p<0.001$), and for the zinc turbidity, 3.33 ($p<0.01$). The difference in zinc turbidity in Series 1 was even larger. The tendency toward lower thymol test values occurred both at *p*H 7.55 and *p*H 7.80. Refrigeration prolonged for 120 hours caused a further decrease in both thymol and zinc turbidities.

A frequency distribution of the data (Table 2) shows that about half of the zinc turbidity and two-thirds of the thymol turbidity measurements changed less than 0.4 units. Only 1 of the 60 thymol

Table 1. Effects of Storage on Certain Hepatic Tests (Mean Values)

		Turbidity Tests (units)				*Bilirubin (mg./100 ml.)*	
		Thymol		*$ZnSO_4$*	*Phenol*		
Series	*Sera*	*pH 7.80*	*pH 7.55*	*pH 7.50*	*pH 5.2*	*1-minute*	*Total*
1		*N=30*	*N=30*	*N=30*	..	*N=31*	*N=31*
	Separated fresh	2.35	3.06	6.48	..	0.07	0.39
	Separated and refrigerated	2.04[a]	2.90	5.64[a]	..	0.09	0.36
2		..	*N=30*	*N=50*	*N=40*	*N=30*	*N=30*
	Separated fresh	..	2.83	5.62	9.26	0.61	1.34
	Separated and refrigerated	..	2.46[a]	5.34[a]	13.45[a]	0.55	1.22
3		*N=27*	*N=27*	*N=25*	*N=18*		
	Separated fresh	3.46	4.92	6.53	18.94	..	..
	Refrigerated with cells	3.33	4.97	6.47	18.37[a]	..	..
4		..	*N=30*	*N=50*	*N=10*	*N=29*	*N=29*
	Separated anaerobic fresh	..	3.14	6.36	8.9	0.96	1.95
	Separated anaerobic refrigerated	..	3.19	6.81[a]	12.5[a]	0.90	1.78[a]

[a]Statistically significant difference, $p < 0.05$.

Table 2. Effects of Storage on Certain Hepatic Tests (Frequencies)
Series 1 & 2

	Thymol test, pH 7.55	*$ZnSO_4$ turbidity, pH 7.50*	*Phenol turbidity, pH 5.2*		*Serum Bilirubin*	
					1°	*TB*
	N=60	*N=80*	*N=40*		*N=61*	*N=61*
Difference (units)				*Difference[a] mg./100 ml.*		
Plus						
>0.6	1	5	30	>0.06	11	..
0.4-0.6	1	2	1	0.04-0.06	8	1
0.2-0.4	5	8	..	0.02-0.04	7	6
No Change						
±0.2	23	17	4	±0.02	14	31
Minus						
0.2-0.4	11	11	..	0.02-0.04	8	19
0.4-0.6	8	9	..	0.04-0.06	..	4
0.6-0.8	6	15	..	0.06-0.08	3	..
0.8-1.0	4	4	1	0.08-0.10	2	..
1.0-1.2	1	2	..	0.10-0.12	1	..
1.2-1.4	..	1	..	0.12-0.14	1	..
>1.4	..	6	4	>0.14	6	..

[a]Difference for 1° bilirubin only; changes in total bilirubin are shown in the first column. Read as mg./100 ml.

turbidity readings and 9 of 80 zinc turbidity readings changed by as much as one unit. Nevertheless, changes in the latter were sufficiently large and frequent to suggest that other methods for processing and storing sera should be investigated.

For this reason, in a third series the sera of 27 blood donors, some with evidence of abnormal liver function, were allowed to remain in contact with the clot in the refrigerator overnight. Comparisons of sera so treated and the original fresh sera (Table 1, Series 3) showed that minimal changes had occurred. The small differences in thymol and zinc turbidities were not statistically significant.

It was then observed that certain sera selected at random from among those reaching the laboratory from hospital patients showed smaller changes after refrigeration than expected on the basis of the experience in the first two series. These specimens differed from those previously studied in that many had been protected against uptake of oxygen and loss of carbon dioxide, by the use of mineral oil. The behavior of these "true" as contrasted to "separated" sera was studied by processing the serum anaerobically and storing under mineral oil in the refrigerator. The results are grouped as Series 4.

Sera handled in this way not only failed to show the lowered zinc turbidity described above, but actually gave higher turbidity readings after 18 hours refrigeration. The rise in zinc turbidity was highly significant ($t = 3.81$, $p < 0.001$).

The thymol turbidity readings were not significantly altered.

Thymol and cephalin-cholesterol flocculations were unaffected by refrigeration or mineral oil.

PHENOL TURBIDITY

A marked increase of nearly 50 per cent in phenol turbidity occurred in sera permitted to stand loosely stoppered overnight in the refrigerator. A rise of comparable size occurred in sera handled anaerobically, which excludes change in *p*H as a factor. However, when serum was allowed to remain in contact with the cells refrigerated overnight only a trivial fall in phenol turbidity occurred. Although significant ($t = 2.11$, $p < 0.05$) it was unimportant in that interpretation of the results was not altered. In addition, a record of serum lactescence, due to elevated lipid content of serum (10), was kept in Series 3. Fifty-two per cent of the sera in this group showed a fall in lactescence by visual estimation as a result of storage.

BILIRUBIN

A tendency toward lower values following refrigeration overnight is evident. However in Series 1 and 2, this was not statistically significant. In Series 4, in which blood was collected anaerobically, the fall in total bilirubin exceeded that to be expected by chance ($t = 3.54$, $p < 0.01$). A similar trend in the "1-minute" reading was not significant.

DISCUSSION

The markedly altered behavior of separated serum as compared with that remaining in contact with cells during the storage period probably involves several factors. Important among these is the loss of CO_2 which, in some way, brings about the decrease in reactivity that is the predominant difference between fresh and stored sera. The role of CO_2 in the zinc turbidity test is discussed in a separate paper (11). The small but significant rise in zinc turbidity of sera that have been stored under oil remains unexplained. No evidence has been obtained for believing that a serum-mineral oil combination alters the photometric measurements. Moreover, the progressive

nature of the change in sera which have not had contact with mineral oil excludes this possibility.

Insignificant changes in the thymol and zinc turbidity measurements occurred when clotted blood was refrigerated without removal of the serum. In these circumstances, losses of CO_2 were minimal.

Strikingly higher phenol turbidity values were noted after refrigeration both with "separated" and "true" sera, the latter collected under mineral oil. A change in the physical or chemical state of the lipid, possibly due to the action of enzymes attacking lipoproteins, seems possible. However, when sera were refrigerated in contact with the clot, the phenol turbidities were virtually unchanged. The slight, although statistically significant, decrease noted in sera so treated is too small to affect interpretation of phenol turbidity measurements.

SUMMARY

Decreased thymol and zinc sulfate turbidity values often were observed after separated serum was refrigerated overnight. In some instances the changes were sufficient to affect appreciably the significance of the results. Phenol turbidity tended to increase considerably after refrigeration. Changes in thymol, zinc, and phenol turbidities were largely prevented by allowing serum to remain in contact with the clot during storage.

The use of anaerobic technic in the collection and storage of serum also prevented changes in the thymol turbidity measurements and, to a lesser extent, in the zinc turbidity. However, a large change in phenol turbidity persisted under these conditions.

The changes in zinc turbidity were related to the CO_2 content of the sera being tested.

Serum bilirubin concentration tended to decrease slightly during storage.

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A Simple Turbidimetric Method of Estimating Blood Urea

Hubert Lawrie

THE CLASSIC ESTIMATION OF UREA IN THE BLOOD, while not technically difficult, is time consuming, and a quicker method was sought for an emergency procedure. It has been known for many years that a solution of xanthydrol with urea forms an insoluble precipitate, dixanthylurea. Also that this precipitate dissolves in 50% sulfuric acid to give an intense yellow-colored solution which obeys Beer's law up to certain concentrations. Unfortunately xanthydrol itself gives the same yellow color in sulfuric acid, so the precipitate of dixanthylurea has to be washed free of the xanthydrol reagent before preparing it for the colorimeter.

Beattie (1), in 1928, described a method which involved the repeated washing of the precipitate and the final evaluation of the yellow solution in sulfuric acid. Later, in 1937, Lee and Widdowson (2) elaborated Beattie's work and devised a micro-method which, they claimed, was accurate. This method was tedious and unsuitable for our purpose. Ten years later, in 1947, Engel and Engel (3) reconsidered the whole matter and produced an elegant method, but again it involved repeated washing of a small precipitate and the colorimetric estimation of the sulfuric acid solution. Caraway (4), in 1955, proposed a turbimetric method using the same reaction of xanthydrol and urea but taking the turbidity of the mixture, after five minutes, as a measure of the urea present. A disadvantage of this method is the continuing development of the turbidity after the stipulated 5 minutes. Furthermore the estimation of turbidity by eye, without any standard for comparison, is very difficult and introduces the per-

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Received for publication February 27, 1957.

Table 1. TURBIDIMETRIC METHOD FOR ESTIMATION[a] OF UREA, USING 5% XANTHYDROL IN METHANOL

Original blood urea	*Urea added*	*Turbidimetric estimation*	*Recovery percent*
30	97	120	94
45	95	130	93
110	89	190	90
35	96	135	103
15	98	105	93
40	96	135	99
35	96	125	96
100	90	182	96
40	96	140	103
110	89	210	106
55	95	145	97
55	95	150	100

[a]All values in milligrams of urea per 100 ml.

The urea was added as a solution of 10 mg. urea in 1 ml. of 50% acetic acid.

To 0.9 ml. of serum (or plasma) was added 0.1 ml. of the urea solution. The above are the means of duplicate determinations.

No satisfactory artificial urea standard solution could be devised so the curve was constructed from estimations made on serum (or plasma) whose urea content had been ascertained by the aeration method. It was found, also, that aqueous solutions of urea did not give good recovery figures; hence the use of the 50% acetic acid solution.

sonal element and error. Accordingly the suggested method was modified as described in the following.

REAGENTS AND APPARATUS

50% Acetic acid. Mix equal volumes of glacial acetic acid and distilled water.

5% Xanthydrol. (British Drug Houses 10% xanthydrol in methanol diluted with an equal volume of methanol)

Thoroughly clean and dry 6″ × ¾″ test tubes.

Thoroughly clean and dry pipet for the xanthydrol solution.

Serum, plasma, or protein-free filtrate prepared from equal parts of whole blood and 20% trichloracetic acid.

E.E.L. Colorimeter with dry 8-mm (O.D.) tubes and the red filter No. 205.

The two reagents keep well. No change in the xanthydrol reagent has been found after four weeks if it is stored in a well-stoppered bottle in the dark.

METHOD

Pipet 0.2-ml. of serum or plasma or 0.4 ml. of the protein-free filtrate into a clean, dry, test tube. Add 4 ml. of the 50% acetic acid, or 3.8 ml. if protein-free filtrate has been used. Add 0.4 ml. of 5% xanthydrol reagent, being careful that it does not touch the side of the tube as it creeps up glass.

Mix by gentle shaking and let stand, at room temperature, for fifteen minutes.

Transfer to a cuvet and read in the colorimeter, using the red filter No. 205.

If a high blood urea is suspected then dilute the specimen 1:1, 1:2, 1:4 etc. with normal saline and use 0.2 ml. of the diluted fluid.

Test tubes and colorimeter tubes can be quickly cleaned with 25% to 50% sulfuric acid and water.

It is most important that all tubes and pipets are quite dry before use, since xanthydrol is precipitated from methanol by water so that high readings would result if moisture were present.

RESULTS

A series of trials was made with bloods of known urea content of from 44 to 406 mg. per 100 ml., and it was found that the turbidity in-

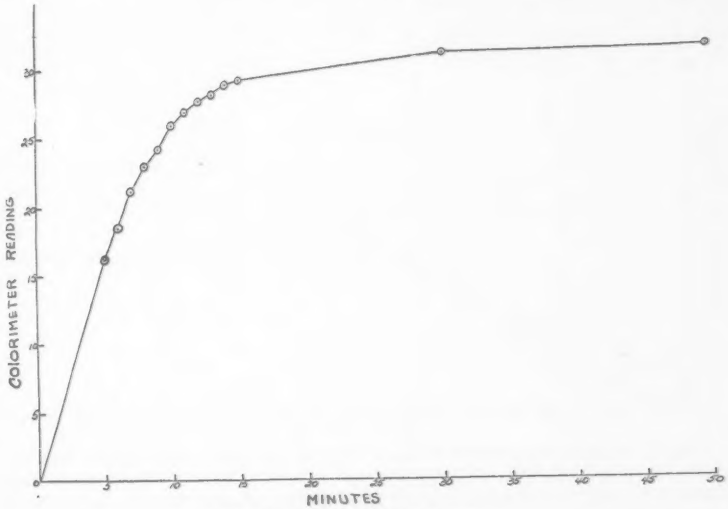

Fig. 1. Time-turbidity curve.

creased steadily after 5 minutes but that the increase became so small at 15 minutes that the manipulation of the specimen and slight delay in reading introduced no significant error. The time-turbidity curve (Fig. 1) became almost flat at 15 minutes, no matter what the concentration of urea so long as it was below 250 mg. per 100 ml.

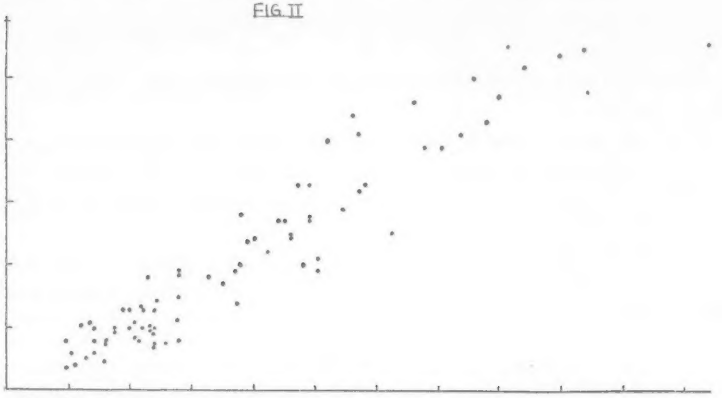

Fig. 2. Scattergram of 100 specimens showing range of urea concentrations examined (mg./100 ml.).

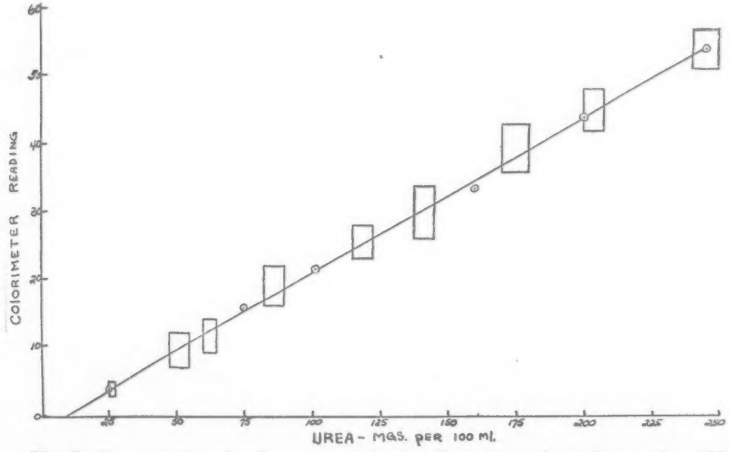

Fig. 3. Concentration-absorbance curve showing that mean values of more than 100 determinations of various concentration levels follow the Beer-Lambert law. Blocked areas show range of galvanometer readings with the mean value for that concentration area falling on the line.

The development of turbidity was not affected by temperature in the range 15° to 37°, or by excessive amounts of either glucose or cholesterol in the blood. Barbiturates or sulfa-compounds present in the specimen gave no interference. It was found, however, that badly hemolysed specimens gave unreliable readings and were best discarded.

One hundred bloods have been examined, with urea contents of from 25 to 760 mg. per 100 ml., and it has been found that up to 250 mg. per 100 ml. the method gives results reliable enough for clinical use. Figure 2 is the scattergram of these estimations. The urease digestion and aeration method of Van Slyke and Cullen (5) was always carried out as a check. In the majority of cases the turbidity reading was within 5% of the value as determined by aeration. Figure 3 shows the spread of urea values at points along the colorimeter scale. The test has proved particularly useful in cases of suspected uremia, for with levels of 100 mg. per 100 ml., and over, a definite turbidity develops within the first minute after the addition of the xanthydrol reagent.

SUMMARY

A rapid turbidimetric method has been described for estimating urea in serum, plasma, or protein-free filtrate. The method uses a xanthydrol reagent and 50% acetic acid, and measurement of the degree of turbidity produced in 15 minutes in a colorimeter using a red filter. Blood urea values of 100 mg. per 100 ml. and over are indicated, clearly, in less than 1 minute.

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Microtest for Mucopolysaccharides by Means of Toluidine Blue

With Special Reference to Hyaluronic Acid

Nelly Blumenkrantz

ALTHOUGH A VAST AMOUNT OF LITERATURE has been published in recent years on the subject of hyaluronic acid and hyaluronidase, owing to the importance of a biologic function which has been ascribed to these two substances, no satisfactory method yet exists for the determination of minute quantities of hyaluronic acid or other mucopolysaccharides.

Various mucopolysaccharides form stable dispersions in water or buffer solutions. The stability of such suspensions can be destroyed by neutralizing the negative charges of the particles with charges of opposite sign.[1] It has been possible in the present study to utilize the alkaline dye toluidine blue for this purpose. In the presence of the dye, hyaluronic acid and other mucopolysaccharides produce colored precipitates of characteristic morphology. Advantage has been taken of these properties to develop a microtest for the detection of minute quantities of hyaluronic acid and related mucopolysaccharides.

MATERIALS AND METHODS

1. *Buffer solutions.* *p*H 1.0-2.2: Clark & Lubs buffers, HCl-KCl (2); *p*H 3.1-6.22: Michaelis buffers, acetic acid-sodium acetate (3); *p*H

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Received for publication April 8, 1957.

The author wishes to express her indebtedness to Prof. I. C. Michaelson for his helpful advice and guidance throughout this work, to Prof. E. Katchalsky, Prof. S. Hestrin, and Dr. M. Wolman for helpful criticism and to the Arnold Reuben Fight for Sight Fund of the National Council to Combat Blindness, Inc., New York, for financial assistance.

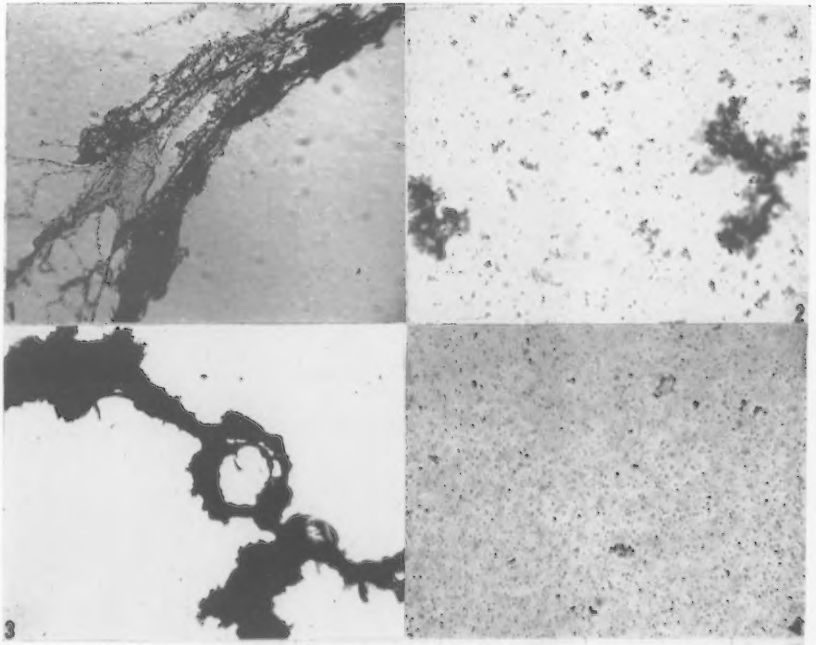

Fig. 1. Filamentous precipitate formed by the interaction of hyaluronic acid and toluidine blue (× 56). **Fig. 2.** Granular precipitate formed when the hyaluronic acid toluidine blue interaction product is stirred with a glass rod in the process of formation (× 150). **Fig. 3.** Precipitate formed by the interaction of toluidine blue with heparin solution, *p*H 2 (× 150). **Fig. 4.** Precipitate formed by the interaction of toluidine blue with chondroitin sulfuric acid, *p*H 2 (× 150).

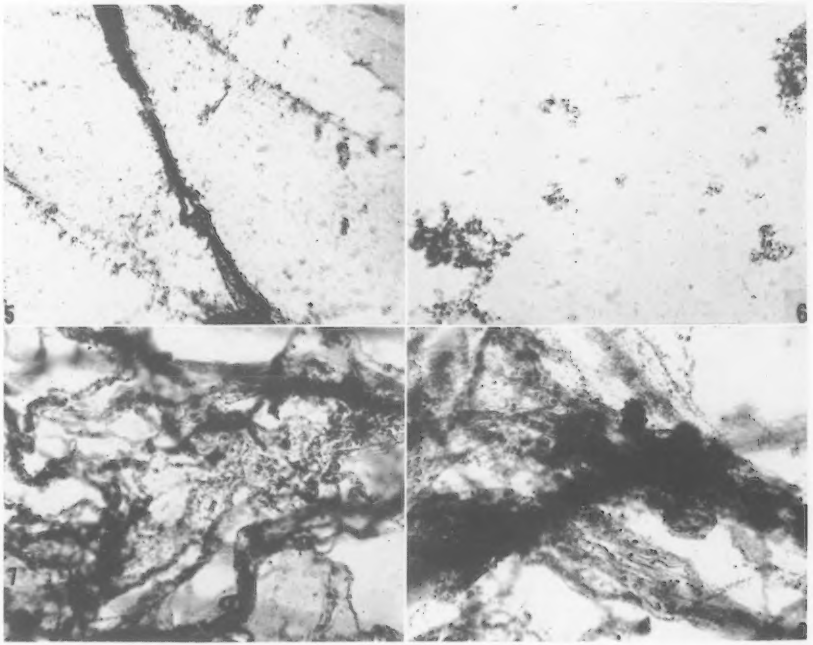

Fig. 5. Precipitate formed by the interaction of toluidine blue with ox vitreous humor (× 56). **Fig. 6.** Precipitate formed by the interaction of toluidine blue with human normal vitreous humor (× 150). **Fig. 7.** Precipitate formed by the interaction of toluidine blue with myopic vitreous humor (× 150). **Fig. 8.** Precipitate formed by the interaction of toluidine blue with subretinal fluid (× 150).

7.8-10.0: Clark & Lubs buffers, boric acid, KCl-NaOH mixtures (2). Above *p*H 10.0, 0.01N NaOH was added to bring the solution to the desired *p*H.

2. *Heparin* (Hynson, Westcott & Dunning, Inc., Baltimore, Md.). Solutions containing 500 μg. per ml. were made up in the above series of buffers, and solutions ranging from 20 to 100 μg. per ml. were made up at *p*H 6.

3. *Chondroitin sulfuric acid* (Nutritional Biochemicals Corp., Cleveland, Ohio). Solutions containing 500 μg. per ml. were made up in the above series of buffers, and solutions ranging from 20 to 100 μg. per ml. were made up in buffer at *p*H 6.

4. *Hyaluronic acid* (human umbilical cord, Worthington Biochemical Co., Freehold, N. J.). Solutions containing 500 μg. per ml. were made up in the above series of buffers, and solutions containing 1.0, 10, 20, 30, 40, 50, and 100 μg. per ml. were made up in M/10 acetate buffer, *p*H 6.

5. *Hyaluronidase* ("Hyalase", Benger Laboratories Ltd., Cheshire, England). The contents of each 1000-unit ampule were dissolved in 1 ml. of M/10 acetate buffer, *p*H 6.

6. *Dye* (George T. Gurr, London). Toluidine blue (dimethyltolutioninechloride) was made up in 0.1 per cent solution in distilled water.

PROCEDURE

The reaction between mucopolysaccharide and dye is carried out by placing 1 drop of dye on a microscope slide and mixing with an equal quantity of buffered mucopolysaccharide test solution. A precipitate is obtained. The type of precipitate formed depends on the manner in which the 2 drops are mixed on the slide. Mixing by tilting the slide gently back and forth produces a precipitate consisting of elongated filaments and fibers (Fig. 1), but if the drops are mixed by stirring with a glass rod, which disrupts the fibers in the process of formation, a characteristic coarsely granular precipitate is obtained (Fig. 2). It is convenient to examine the slide for a precipitate about 10 minutes after the drops are mixed.

Precipitates of the same general type, but differing in form and color, were also obtained with heparin (Fig. 3), and chondroitin sulfuric acid (Fig. 4). Similarly, metachromatic precipitates were obtained with various optic fluids such as ox vitreous humor (Fig. 5), human normal and pathologic vitreous humor (Figs. 6 and 7), aque-

ous humor, subretinal fluid (Fig. 8), and extracts of lens and cornea. With fluids, the reaction was carried out by adding 1 drop of toluidine blue to 1 drop of test material diluted with one drop of buffer solution at *p*H 6. If a dry material such as conjunctival smears was tested, it was first dissolved in a minimum quantity of buffer, *p*H 6, and then mixed with an equal volume of toluidine blue on the slide. The addition of one drop of human serum to an equal quantity of toluidine blue caused the formation of a fine, powdery blue precipitate readily distinguishable from the filamentous or coarsely granular precipitate obtained in the reaction between hyaluronic acid and toluidine blue. Fibrin and toluidine blue produced a precipitate composed of blue globules distinctly different from the filamentous precipitates formed by the same dye with hyaluronic acid.

EFFECT OF HYALURONIDASE

Test tubes containing one drop of hyaluronic acid at a concentration of 200 μg. per ml. were incubated for various lengths of time at 37° with 50, 100, 200, and 1000 units of hyaluronidase. One drop of toluidine blue was added to each test tube. Increasing the concentration of hyaluronidase produces the same effect as prolonging the time of incubation—diminution of the length of the characteristic precipitate and finally no precipitation. Hyaluronic acid depolymerized to oligosaccharides as a result of enzymatic action produces no precipitate with toluidine blue. The formation of a precipitate between the various biologic fluids or extracts of the eye mentioned above and toluidine blue could similarly be precluded by prior incubation with hyaluronidase.

SENSITIVITY OF METHOD

The minimum quantity of hyaluronic acid detectable by the present method was shown by serial dilution to be 0.5 μg. (10 μg./ml.). In very dilute solutions of hyaluronic acid, a microscope is necessary to detect the precipitate.

Robertson, Ropes, and Bauer (4) have shown that hyaluronic acid in the presence of proteins precipitates in a fibrous clot when the solution is acidified with acetic acid. To compare the sensitivity of the present method with that of Robertson *et al.*, protein-containing human vitreous humor was tested in various dilutions with the reagents employed in the two methods. The results are shown in Table 1. No precipitate could be detected by the modification of Robertson's

Table 1. Comparison of Hyaluronic-acid-Detecting Methods

		Presence of precipitate		
		With acetic acid		*With toluidine blue*
Tube no.	*Dilution*	*Macroscopic (Robertson)*	*Microscopic*	
1	1:2	+	+	+
2	1:4	±	+	+
3	1:8	−	+	+
4	1:16	−	±	+
5	1:32	−	−	+
6	1:64	−	−	+

Test substance: human vitreous humor, serially diluted with buffer, *p*H 6. The reactions were carried out with one drop of the dilutions mentioned above.

method under the microscope at dilutions of vitreous humor higher than 1:16, whereas the use of toluidine blue enabled the detection of positive-reacting material in vitreous humor in dilutions a high as 1:64.

EFFECT OF pH

The effect of *p*H on the reaction between mucopolysaccharides and dye was studied by mixing 1 drop of a solution of mucopolysaccharide in buffer solution at various *p*H's over the range from *p*H 1-14 with an equal quantity of toluidine blue dissolved in buffer at an identical *p*H. It was found that while all mucopolysaccharides tested have an optimum zone of precipitation between *p*H 6-10, heparin (a stronger acid) produces a precipitate at a *p*H as low as 1.4, whereas chondroitin sulfuric acid fails to give a precipitate at a *p*H lower than 2, and hyaluronic acid at a *p*H lower than 3.3. The characteristic colors of the precipitates at *p*H 6 are recorded in Table 2.

Table 2. Color of Precipitate Obtained on Mixing One Drop of Various Mucopolysaccharides (200 μg. per ml.) with One Drop of Toluidine Blue in Buffer Solution *p*H 6

Mucopolysaccharide	*Precipitate color*
Heparin	Purple
Chondroitin sulfuric acid	Purple-violet
Hyaluronic acid	Dark violet

DISCUSSION

The present investigation was undertaken when it was found to be impossible to detect hyaluronic acid in low concentrations in certain specimens of normal and pathologic eye fluids by any existing method. The quantitative method proposed by Tolksdorf (5), based on the amount of turbidity produced in hyaluronic acid solution at an acid *p*H by the addition of protein as read in a photoelectric colorimeter, can only be used when concentrations of hyaluronic acid greater than 32 μg. per ml. are present. Tests conducted on the sensitivity of the modified Robertson, Ropes, and Bauer method (4) showed that it was impossible to detect hyaluronic acid in concentrations lower than 30 μg./ml. in the presence of horse serum, or in vitreous humor in humor dilutions greater than 1:16, even when a microscope was used. The method presented in the present paper utilizing toluidine blue permits as little as 0.5 μg. of hyaluronic acid to be detected and affords a positive reaction with vitreous humor at a humor dilution of 1:64. By means of the present method and with pure hyaluronic acid at a concentration of 10 μg. per ml. (we use only 1 drop), it is possible to get positive results.

SUMMARY

A micromethod is presented for the detection of hyaluronic acid when as little as 0.5 μg. of material are present. The method is simple and applicable as well to some other mucopolysaccharides.

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Improved Method for the Colorimetric Determination of Glutamic-Oxalacetic Transaminase Activity

Fred L. Humoller, Joseph M. Holthaus, and John R. Walsh
With the technical assistance of *Ethel McCann*

RECENTLY CABAUD ET AL. (1) HAVE PUBLISHED A METHOD for the quantitative determination of serum glutamic-oxalacetic acid transaminase (GOT). Their method is an adaptation of the Friedemann and Haugen (2) and the Tonhazy *et al.* (6) method for pyruvic acid and tissue transaminase, respectively. In the Cabaud method the oxalacetic acid formed as the result of transamination is catalytically converted to pyruvic acid. This substance is then converted to its 2,4-dinitrophenylhydrazone, which is then estimated colorimetrically. The method as described in the literature is rapid and appears to be suited for use in clinical laboratory practice. However, unless all conditions involved in the determination are well understood and controlled, serious errors may occur particularly when the determination is carried out routinely. The present report describes a modification which minimizes these errors and thus increases the usefulness of the method.

EXPERIMENTAL

REAGENTS

Aspartate-ketoglutarate-phosphate buffer: Dissolve 2.66 Gm. of aspartic acid, 0.2 Gm. of alpha-ketoglutarate and 2.0 Gm. of KH_2PO_4 in water. Adjust to *p*H 7.4 and dilute to 100 ml.

Aniline citrate reagent: Dissolve 5 Gm. of citric acid in 5.0 ml. of water. To this solution add 5.0 ml. of aniline. The reagent should be

From the Medical Research Laboratory, Veterans Administration Hospital, Omaha, Nebr.
Received for publication June 27, 1957.

prepared fresh before use as polymerization occurs on standing.

Trichloroacetic acid (TCA) 100% w/v: Dissolve 100 Gm. of TCA in water and dilute to 100 ml.

DNPH reagent: Dissolve 200 mg. of 2,4-dinitrophenylhydrazine (DNPH) in 20 ml. of conc. HCl and dilute to 100 ml. with water.

Toulene, reagent grade: Place 100 ml. of high purity toluene in a separatory funnel. Shake with 20 ml. of distilled water and discard the water layer.

Sodium bicarbonate solution 5%.

Aqueous sodium hydroxide, 10%.

PYRUVIC ACID STANDARDS

Dissolve 100 mg. of pyruvic acid or 126 mg. of high purity sodium pyruvate in 2% potassium monophosphate buffer, *p*H 7.4. Dilute to 200 ml. From this stock solution prepare a series of working standards by diluting 0.5, 1.0, 2.0, and 3.0 ml. to 10 ml. with the phosphate buffer. These standards will contain then 25, 50, 100, and 150 μg. of pyruvic acid per 1.0 ml. of solution, respectively. They are carried through whole procedure and the final absorbances are plotted on graph paper against the concentrations. From this standard curve the color intensity of the serum samples can be converted to μg. of pyruvate formed per ml. of serum.

PROCEDURE

To each of two test tubes 0.5 ml. of serum and 0.5 ml. of the aspartate-ketoglutarate-phosphate buffer is added and followed immediately by 2 drops of TCA solution and 1 drop of the aniline citrate solution to one of the tubes to serve as serum control. Similarly a series of tubes containing 0.5 ml. samples of the standard pyruvic acid solutions are prepared and all tubes are incubated for exactly 20 minutes at room temperature (24-26°). At the end of this time the reaction is stopped by adding 2 drops of TCA and 1 drop of aniline citrate to the second group of tubes. After mixing, the tubes are allowed to stand for 20 minutes at room temperature for conversion of the oxalacetate to pyruvate. Now 0.5 ml. of the DNPH reagent are added to each tube, the contents of the tubes are thoroughly mixed and allowed to stand for 3-5 minutes. Then 2 ml. of toluene are added to each tube which is then thoroughly shaken to extract the pyruvic acid hydrazone into the toluene layer. After a clear separation of the layers has been obtained, a 1.0 ml. aliquot of the toluene layer is with-

drawn from each tube and transferred to a test tube containing 5.0 ml. of 5% sodium bicarbonate solution. The contents of each of these tubes are thoroughly shaken to extract the hydrazone into the bicarbonate solution, leaving the unreacted DNPH in the toluene layer. Then 4.0 ml. of the clear aqueous layer is transferred to other test tubes. After adding 1.0 ml. of 10% NaOH to each of the tubes, and thorough mixing, the color intensity is measured in a photolometer or spectrophotometer at 440 mμ. The percentage transmittance or absorbance values are then converted into μg. of pyruvic acid or units of transminase activity by use of the standard curve.

INTERCONVERSION OF UNITS

By definition (3) the spectrophotometric unit is equivalent to a decrease in optical density of 0.001 (units) per minute, per ml. of serum at 340 mμ. Using the molar absorbancy index value of $DPNH_2$ at 340 mμ of 6.22×10^3 (5) it can be shown that under the conditions of the experiment a decrease in absorbance of 0.001 is equivalent to the oxidation of 4.83×10^{-4} μM $DNPH_2$. The chromatographic unit by definition (4) is the formation of 1 μM glutamic acid per hour incubation for 1.0 ml. of serum. Hence spectrophotometric units $\times$ 60 $\times$ 4.83 $\times$ 10^{-4} = chromatographic units, or conversely $\frac{\text{chromatographic units}}{0.029}$ = spectrophotometric units.

A pyruvic unit by definition (1) is the formation of 1.0 μg. of pyruvic acid under the condition of the procedure or to the formation of 5.8×10^{-4} μM per minute incubation for 1.0 ml. of serum. Hence pyruvic acid units $\times$ 1.2 = spectrophotometric units.

APPARATUS

All absorbance measurements were made with the Beckman spectrophotometer Model DU.

RESULTS

The pyruvic acid method for measuring the activity of the transaminase system is based upon reactions 1 and 2. The oxalacetic acid formed in reaction 1 is converted to pyruvic acid by the catalytic action of aniline citrate. The pyruvic acid thus formed reacts with DNPH to form a hydrazone which is intensely colored in strongly alkaline solution. It can thus be estimated colorimetrically and serve as an index of the transaminase activity. However, as shown in reaction 3, along with the pyruvate hydrazone the reaction mixture will

also contain some ketoglutarate hydrazone and unreacted DNPH. To be specific for pyruvic acid, the method must therefore include provisions for the quantitative separation of the pyruvate hydrazone from the other two substances, since they too form highly colored solutions in the presence of strong alkali. In the proposed method interference by unreacted DNPH is nearly completely eliminated through the final extraction of the hydrazone from toluene into bicarbonate. This is well illustrated in an experiment the results of which are summarized in Table 1. To each of two tubes were added 1.5 ml. of the DNPH reagent (0.1%) and 4.5 ml. of 2% phosphate buffer, *p*H 7.4. The mixture was thoroughly shaken with 6.0 ml. of toluene and centrifuged for 5 minutes to obtain a sharp separation of the layers. The absorbances of the buffer and toluene layers were then measured. After that the toluene solution was shaken with an equal volume of 5% bicarbonate solution and the absorbances of these two solutions measured. As Table 1 shows, there is about equal distribution of DNPH between the buffer layer and the toluene, but practically none of the DNPH is extracted into the bicarbonate. Hence in the proposed method the possible interference of unreacted DNPH is eliminated, whereas without the bicarbonate extraction about one-half of whatever unreacted DNPH is left in reaction 3 will appear in the final solution and contribute to the color of the pyruvate hydrazone.

It can readily be demonstrated that toluene selectively extracts the hydrazone of pyruvic acid from a mixture of pyruvate and ketoglutarate hydrazones. Table 2 summarizes the results obtained in an experiment in which 100 μg. quantities of pyruvic acid and varying amounts of ketoglutarate were allowed to react with DNPH for 5 minutes. When a nonselective solvent such as ethyl acetate is used, the absorbance of the final solution increases proportionally with the amount of ketoglutarate added. When toluene is used to extract the

Table 1. DISTRIBUTION OF DNPH BETWEEN BUFFER, TOLUENE, AND BICARBONATE

	Absorbance[a]	
	#1	#2
Reaction mixture		
Buffer layer after extraction	.178	.183
Toluene layer	.175	.182
Toluene layer after extraction with $NaHCO_3$	.177	.187
Bicarbonate layer	.018	.010

[a]Results of duplicate experiments.

Table 2. SEPARATION OF PYRUVIC ACID HYDRAZONE FROM THE ALPHA-KETOGLUTARATE HYDRAZONE

Amount of alpha-ketoglutarate added (μg.)	*Absorbance*[a]	
	Ethyl acetate extracted[b]	*Toluene extracted*[b]
0	0.463	0.455
60	0.508	0.403
120	0.565	0.484
180	0.618	0.485

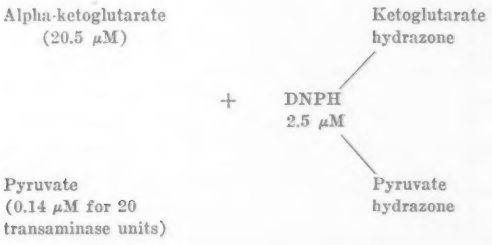
[a]All tubes contained 100 μg. pyruvic acid. Reaction time 5 minutes.
[b]Averages of duplicates.

hydrazones no such increase in absorbance is observed. This experiment thus confirms the merit of toluene as a selective extractant of the pyruvate hydrazone, but it also shows that significant amounts of ketoglutarate hydrazone are formed in a reaction period of 5 minutes' duration. The competition of ketoglutarate with pyruvate for the available DNPH is therefore a factor which must be considered. In reaction 2 the quantitative relationship of this competition, as it exists under the conditions of the original method, is illustrated.

REACTION 1

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \underset{\longleftarrow}{\xrightarrow{\text{transaminase}}} \text{oxalacetic acid} + \text{glutamic acid}.$$

REACTION 2



Thus with 0.5 ml. of normal serum 20.5 μM of ketoglutarate compete with 0.1 μM of pyruvate for the available 2.5 μM of DNPH. Even though it can be readily shown that the reaction between DNPH and pyruvate proceeds at a considerably faster rate than that between

ketoglutarate and DNPH, the concentration ratio of 1:200 of these two keto acids raises the question that the presence of ketoglutarate may interfere with quantitative formation of the pyruvate hydrazone. To determine the extent of this possible interference an experiment was carried out, the results of which are summarized in Fig. 1. In this experiment four series of test tubes were prepared containing increasing amounts of pyruvic acid. In one series the pyruvic acid was reacted with DNPH under conditions as specified by Cabaud (1)—addition of 0.6% ketoglutarate aspartate buffer and 0.1% DNPH and development of the final hydrazone color in alkaline alcohol-toluene solution. The results obtained are shown in Curve 3. In Curve 4 are shown the results when another series of tubes was carried through the same procedure except that the pyruvate hydrazones formed were extracted into 5% bicarbonate solution and the final color developed in an aqueous medium. The third series of tubes was carried through the hydrazone reaction in the absence of ketoglutarate. The pyruvate hydrazones were extracted into 5% bicarbonate solution and the final color developed by aqueous alkali (Curve 1). This series should represent the maximum pyruvate hydrazone formation which can occur under these conditions, and the differences in absorbance recorded in Curve 4, on the one hand, and Curve 1, on the other hand, represent

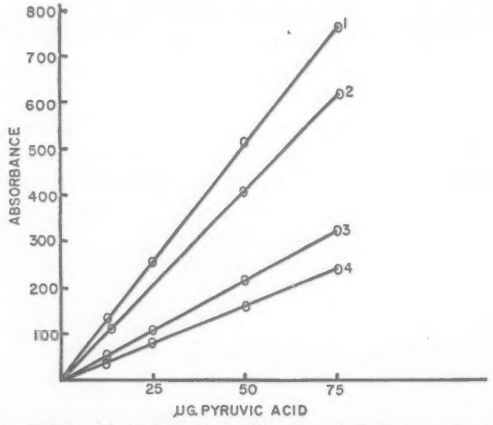


Fig. 1. Effect of ketoglutarate on the pyruvate hydrazone formation.

Curve 1. Phosphate buffer alone. No ketoglutarate. 0.2% DNPH. *Curve 2.* Proposed method. 0.2% Ketoglutarate. 0.2% DNPH. *Curve 3.* Original method. 0.6% Ketoglutarate. 0.1% DNPH. *Curve 4.* Bicarbonate extraction. 0.6% Ketoglutarate. 0.1% DNPH.

the interference of ketoglutarate with pyruvate hydrazone formation. The final series of tubes was carried through the hydrazone reaction under the conditions specified by the proposed method—0.2% ketoglutarate aspartate buffer, 0.2% DNPH, extraction into 5% bicarbonate solution, and final color development in aqueous alkali (Curve 2). It will be noticed that in this series the recorded absorbances, while not actually coinciding with those in Curve 1, approach them very much closer than those of Curve 4. Thus in the proposed method the final results obtained are more nearly a function of the actual amount of pyruvic acid formed in the transamination reaction and are influenced less by the presence of ketoglutarate than they are under the conditions of the original method. Reduction in the amount of ketoglutarate obviously reduces the effective range of the method but it is felt that extremely high serum transaminase activity is observed so rarely that in these few cases the test could be repeated using a smaller amount of serum.

DISCUSSION

Three methods have been proposed for the quantitative estimation of transaminase activity. These are the spectrophotometric (3) the chromatographic method (4), and the pyruvic acid dinitrophenylhydrazone method (1). Each of these methods is capable of giving precise results in the hands of competent workers and, as Table 3 shows, when applied to the same sera the results obtained are comparable. As the results of hundreds of transaminase determinations carried out in this laboratory (7) by these three methods it was decided that the colorimetric method of Cabaud *et al.* was the most suitable method of the three for routine determination, particularly if the modifications described in this report were made part of the procedure. The introduction of the step involving the extraction of the pyruvate dinitrophenylhydrazone with aqueous bicarbonate removes one possible source of error. In the proposed method the final color measured is due solely to the presence of the pyruvic acid dinitrophenylhydrazone and the interference of unreacted DNPH is removed rather than compensated.

Decreasing the amount of alpha-ketoglutarate added to the transaminase system reduces the range of the method somewhat but the results of the hydrazone reaction represent more nearly the true amount of pyruvate present (Fig. 1, Curves 1 and 2) than those obtained in the presence of higher concentrations of ketoglutarate.

Table 3. TRANSAMINASE ACTIVITY OF HUMAN SERA BY THREE DIFFERENT METHODS

Human sera[a]	*Spectrophotometric*	*Chromatographic*[b]	*Pyruvic acid*[b]
G. F.	35	17.3	28.7
B. H.	12	10.3	10.3
N. K.	20	10.3	17.2
G. D.	20	44.8	29.2
A. R.	50	34.5	30.8
H. R.	50	41.5	65.5
T. H.	14	38	34.3
C. T.	16	27.6	20.8
T. W.	14	31.1	21.1
N. C.	36	13.6	18.7
H. H.	63	34.5	40.6
R. C.	20	13.9	14.5
R. K.	10	34.5	17.4
L. S.	20	6.9	15.7
G. V.	10	13.8	8.2
F. W.	14	31.1	26.0
W. K.	20	10.3	18.8
E. L.	16	24.2	12.4
J. P.	18	20.7	6.8
F. R.	16	11.0	12.4

[a] Hospital patients selected at random.
[b] All values converted to spectrophotometric units. See text.

SUMMARY

A modification of the Cabaud *et al.* method (1) for the determination of SGOT activity is described. The proposed modification consists of the elimination of the interference of unreacted DNPH by extracting the pyruvate dinitrophenylhydrazone with aqueous bicarbonate. The proposed method also employs a lower concentration of alpha-ketoglutarate, thus reducing its competition for the available DNPH.

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Absorbance of Various Protein-Free Filtrates of Serum

Richard J. Henry and Sam Berkman

THE PREPARATION of a protein-free filtrate is one of the most common preliminary steps in the determination of a substance in serum or other body fluid. Such filtrates in practically all instances appear colorless to the eye, and it has been tacitly assumed, therefore, that any nonspecific absorbance which may be present is too small to

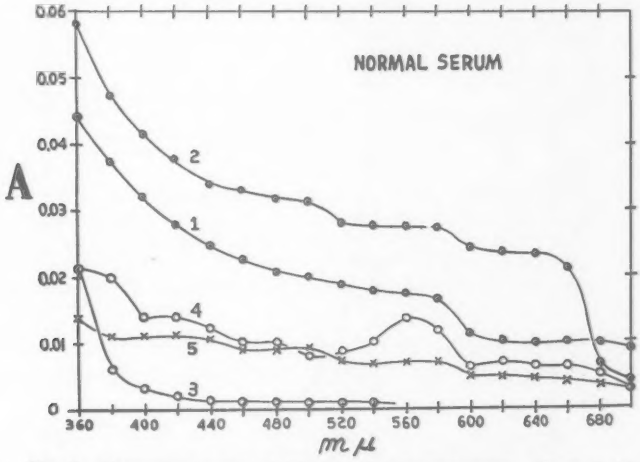

Fig. 1. Absorption curves of filtrates of a normal serum. *Curve 1*, trichloroacetic acid filtrate (1:10), *p*H 0.84. *Curve 2*, trichloroacetic acid filtrate (1:10), *p*H 11. *Curve 3*, tungstic acid filtrate (1:10), *p*H 4.5. *Curve 4*, tungstic acid filtrate (1:10), *p*H 11. *Curve 5*, zinc hydroxide filtrate (1:10), *p*H 7.

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Received for publication September 23, 1957.

significantly contribute to any specific absorbance developed by a color reaction carried out on the filtrate.

Gubler *et al.* (1), in a study of the photometric determination of serum copper with diethyldithiocarbamate, noted that trichloroacetic acid filtrates of serum previously treated with hydrochloric acid possessed a variable but significant absorbance at 440 mμ, the wavelength employed for measurement of the copper carbamate color. They also noted that this background absorbance was increased if the precipitation of proteins was carried out at elevated temperatures. It was decided to further investigate this phenomenon by obtaining

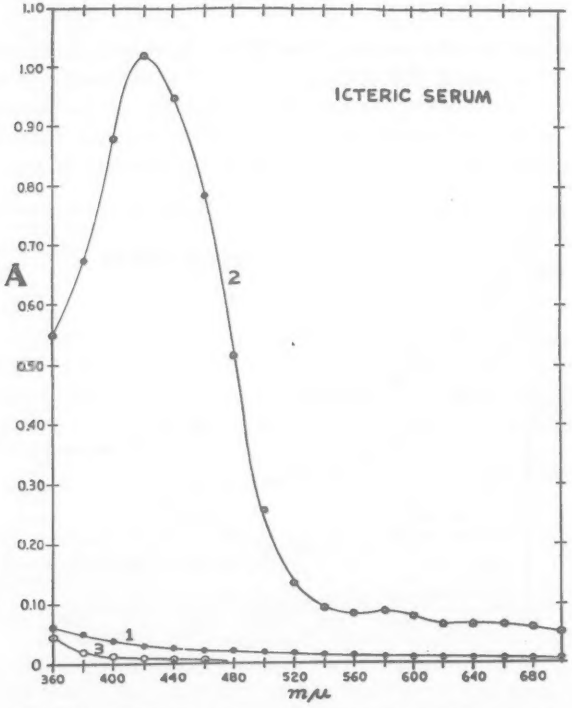

Fig. 2. Absorption curves of filtrates of an icteric serum. Bilirubin concentration of serum 7.3 mg. per 100 ml. *Curve 1*, trichloroacetic acid filtrate (1:10), *p*H 0.83. *Curve 2*, trichloroacetic acid filtrate (1:20), *p*H 11. *Curve 3*, tungstic acid filtrate (1:10), *p*H 4.4.

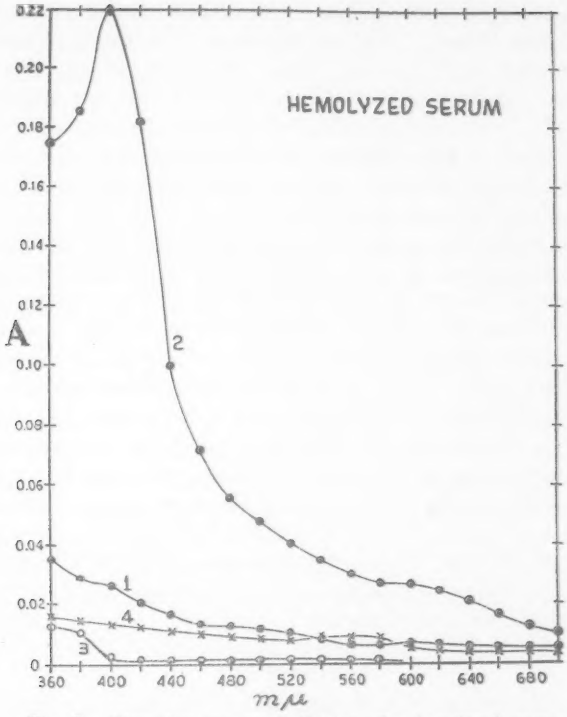

Fig. 3. Absorption curves of filtrates of a hemolyzed serum. Hemoglobin concentration of serum 200 mg. per 100 ml. *Curve 1,* trichloroacetic acid filtrate (1:10), pH 0.89. *Curve 2,* trichloroacetic acid filtrate (1:20), pH 11. *Curve 3,* tungstic acid filtrate (1:10), pH 4.4. *Curve 4,* zinc hydroxide filtrate (1:10), pH 7.1.

absorbance curves of three commonly employed protein-free filtrates prepared from samples of normal, icteric, and hemolyzed sera.

EXPERIMENTAL

Figures 1, 2, and 3 show typical absorbance curves obtained for 1:10 trichloroacetic acid, tungstic acid, and zinc hydroxide filtrates of normal, icteric, and hemolyzed sera, respectively. All filtrates were read against water on a Beckman Model DU spectrophotometer. The final concentration of trichloroacetic acid was 5 per cent, the technic of preparing the tungstic acid filtrate was that of Folin and

Wu, with half-quantities of reagents, and the zinc hydroxide filtrate was the usual Somogyi filtrate, obtained with the quantities of reagents usually employed for whole blood. In addition, curves are shown for trichloroacetic and tungstic acid filtrates brought to an alkaline *p*H. Visually, all filtrates were colorless and exhibited no Tyndall effect, except that the trichloroacetic acid filtrates of the icteric and hemolyzed sera became green and yellow, respectively, upon rendering them alkaline.

Similar curves were run on many sera and the background absorbance was found to vary considerably. For example, the curves of trichloroacetic acid filtrates of clear normal sera were frequently much lower than the example shown in Curve 1, Fig. 1, and on occasion the curve obtained after addition of alkali was considerably higher than Curve 2 of Fig. 1. In every case studied, tungstic acid and zinc hydroxide filtrates had background absorbances lower than those seen in the trichloroacetic acid filtrates. Zinc hydroxide filtrates showed little or no increase upon acidification or alkalization. Curves for the icteric serum of Fig. 2, obtained with zinc hydroxide and alkalization of the tungstic acid filtrate, fell between Curves 1 and 3 and are not shown. The curve obtained upon alkalization of the tungstic acid filtrate of the hemolyzed serum in Fig. 3 lay somewhat above Curve 3, and is not shown.

DISCUSSION

The positive error of a photometric determination resulting from the nonspecific background absorbance of protein-free filtrates will increase as the absorbance due to the specific color reaction decreases. Furthermore, the error generally appears to increase as the wavelength employed decreases, and is greater with trichloroacetic acid filtrates than with zinc or tungstic acid filtrates. It is easy to see how quite large errors can occur in analyses such as serum iron and copper where low-dilution trichloroacetic acid filtrates are employed and, even with the most sensitive color reagents available, the absorbances obtained on normal sera may be less than 0.1. As suggested by Gubler *et al.* (1), correction for the background absorbance can be made by reading the absorbance before addition of the final color reagent, and subtracting this value from that obtained after color development. The validity of this approach rests on the assumption that the specific and nonspecific absorbances are additive and that the latter is unaltered by the presence of the color reagent—an as-

sumption which may or may not be valid. It is important to note that recovery studies with internal standards are not affected by the presence of background absorbance.

As an example of the magnitude of error which can occur in a determination other than serum iron or copper, the error introduced by the background absorbance of the trichloroacetic acid filtrate of Fig. 1, Curve 1, in the determination of serum inorganic phosphate was determined. Employing the method of Dryer *et al.* (2), using a filter with nominal wavelength at 660 mμ and assuming a serum phosphorus level of 3 mg. per 100 ml., the error was calculated to be about 5 per cent.

SUMMARY

Absorbance curves have been run on protein-free serum filtrates obtained with trichloroacetic acid, tungstic acid, and zinc hydroxide. The background absorbance was highest in trichloroacetic acid filtrates, lowest in zinc hydroxide filtrates, and increased with decreasing wavelength. The possible error produced in photometric determinations by this nonspecific absorbance and means of correction have been discussed.

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Studies with a New Colorimetric Test for Proteinuria

Alfred H. Free, Chauncey O. Rupe, and Ingrid Metzler

DURING THE PAST HALF CENTURY a large number of tests for the detection of protein in the urine have been described. Three of these tests have gained outstanding popularity and are widely used in clinical practice. These are the sulfosalicylic acid test, the nitric acid ring test, and the heat and acetic acid test. Because of the wide usage of these tests as a part of "routine urinalysis" there is a tendency to disregard their shortcomings, but critical consideration of the problem suggests possibilities for improvement.

The present report describes a new type of test for the detection of protein in the urine and presents data on the behavior of the test.

METHODS

GRADATION OF TURBIDIMETRIC METHODS

Procedures for carrying out these tests are subject to wide variation from one laboratory to another. Accordingly, detailed descriptions of the procedures for each method, as carried out in our laboratory, are given in the following paragraphs. In all cases the results were graded as follows in a laboratory brightly lighted with fluorescent light: *Negative,* clear; *trace,* faint turbidity or ring; *Small:* 1+, turbidity or ring easily seen from side, 2+, particles beginning to flocculate, ring barely visible from above; *Large:* 3+, definite flocculent particles, ring easily visible from above, 4+, practically solid, thick opaque ring.

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Presented in part at the 131st Meeting of the American Chemical Society, the Division of Biological Chemistry, April, 1957, Miami, Fla.

Received for publication July 19, 1957.

SULFOSALICYLIC ACID TEST

This test was carried out using Bumintest.[1] This reagent is supplied in tablets which contain 400 mg. of sulfosalicylic acid and 56 mg. of sodium bicarbonate. When the tablets are placed in water they effervesce and rapidly dissolve. The working reagent is made by adding 4 tablets to 30 ml. water to give a final concentration of approximately 5 per cent sulfosalicylic acid. Equal quantities of reagent solution and urine (from 0.5 ml. to 1 ml. of each) are mixed in a small test tube. The tube is shaken gently and the reading made immediately. The amount of protein is indicated by the degree of turbidity.

NITRIC ACID RING TEST

Approximately 1 ml. of concentrated nitric acid is placed in a small test tube. An equal quantity of urine is stratified on top of the acid, and the interface of the solutions observed at 2 or 3 minutes for a ring of white turbidity. The amount of protein present is estimated by the degree of turbidity.

HEAT AND ACETIC ACID TEST

A small narrow Pyrex test tube is two-thirds filled with urine (approximately 5 ml.). The upper portion of the urine is heated to boiling over a micro burner. Three drops of 1 per cent acetic acid are then added and mixed gently with the heated portion of the urine. The upper portion of urine is heated to boiling again. Any turbidity remaining or appearing after acidification and reboiling is due to protein. The amount of turbidity is proportional to the amount of protein present in the urine.

EVOLUTION OF THE NEW COLORIMETRIC METHOD

Sörensen (1), in 1909, noted that the presence of protein in a solution caused certain *p*H indicators to exhibit altered colors from that which they would have at the same *p*H in the absence of protein. This phenomenon has been called "the protein error of indicators" and has caused a considerable amount of difficulty to biochemists attempting to measure the *p*H of body fluids with indicators. In the present color tests this phenomenon has been utilized to detect protein.

A buffer of such a *p*H is employed so that a selected indicator will just barely be on one side of the *p*H range at which it changes color,

[1]Bumintest is a registered trademark of Ames Company, Inc., Elkhart, Ind.

usually the acid side. In the presence of protein the indicator, as a result of its "protein error," will exhibit the color it has on the other side of the *p*H range of color change, usually the alkaline side. This occurs even though the *p*H is maintained constant by the buffer.

In order to increase the sensitivity of the color test a means of concentrating the protein from urine is employed. By using cellulose powder or bibulous paper, which is also cellulose, protein can be adsorbed on the surface of the material and thus be made available in increased concentrations for the color reaction.

From the principles of concentration by adsorption and "protein error of indicators" two forms of the colorimetric test have evolved. One of these, which is a tablet test, has been described by Fonner and Collins (2) and the other, which is a strip test, is herein described.

COMPOSITION AND PROCEDURE FOR ALBUTEST[2]

A tablet consisting of bromphenol blue, salicylate buffer at *p*H of approximately 3, and cellulose is prepared on a mechanical tablet press. The tablet is placed on a clean surface and one drop of urine is placed on the tablet. After the urine drop has been absorbed, two drops of water are placed on the tablet. The color of the top of the tablet is then compared with the colored photograph which is supplied with the direction sheet. The colored photograph shows 4 tablets with reactions representing negative, ± (5-15 mg./100 ml.), 1+ (20-100 mg./100 ml.), and 3+ (100-500 mg./100 ml.).

Positive: If protein is present in the urine sample, a blue-green spot will remain on the tablet surface after addition of the water. The amount of protein present influences the intensity of the blue-green spot.

Negative: If no protein is present, the original color of the tablet will not be materially changed at the completion of the test.

COMPOSITION AND PROCEDURE FOR ALBUSTIX[3]

Thick bibulous filter papers are impregnated with a solution supplying tetra bromphenol blue and citrate buffer at approximately *p*H 3. The impregnated strips are dried in an oven. To perform a test with the strips, the yellow end is dipped in the urine sample or moistened in the urine stream at the time of urination. The color of the dipped end is compared with the color chart supplied with the product. This

[2]Albutest is a registered trademark of Ames Company Inc., Elkhart, Ind.
[3]Albustix is a trademark of Ames Company Inc., Elkhart, Ind.

chart shows a yellow block for negative, and 4 shades of green to blue colors representing protein concentrations of 30, 100, 300, and over 1000 mg. per 100 ml. In tabulating results for comparison with other tests, reactions giving colors less than the 30 mg. per 100 ml. color, but definitely greener than the negative color, are called trace; reactions giving colors matching the 30 or 100 mg. per 100 ml. color blocks or in between the two are called small; and reactions giving a color most closely matching the 300 mg. protein per 100 ml. color block or darker are called large.

EXPERIMENTAL

In order to evaluate the behavior of the new colorimetric tests they were compared with one or more of the turbidimetric methods in several types of experiments. These included comparative studies with different tests on urines from healthy subjects, studies on random urines from hospital patients, and studies on urines with added protein. Testing was carried out by both experienced operators (technicians who were familiar with urinalysis procedures including protein testing) and inexperienced operators (including secretaries, librarians, stenographers, and chemists unfamiliar with clinical testing procedures).

STUDIES ON RANDOM URINES (HEALTHY SUBJECTS AND HOSPITAL PATIENTS)

Table 1 presents data obtained on 1371 urine samples from 804 healthy subjects, and 567 random hospital patients. In this series the colorimetric tablet test was compared with the sulfosalicylic acid test. Both tests were negative in 1219 instances, and both tests were posi-

Table 1. Comparison of Colorimetric Tablet Test with Sulfosalicylic Acid Test
Number of Urines Giving Designated Reaction

	Sulfosalicylic acid test		
	Negative	*Trace*	*Positive*
Tablet test—Negative	1219	61	4
Tablet test—Trace	2	–	–
		85	
Tablet test—Positive	0	–	–
Tests agree (Both positive or both negative)		95.1%	
Trace vs. Negative		4.6%	
Tests disagree (One positive other negative)		0.3%	

tive in 85 instances. Sixty-three urines (4.6 per cent of the total number of urines) gave trace reactions with one test and negative with the other. Nearly all of these were trace reactions with the turbidimetric test and negative with the colorimetric tablet test. Significant disagreement between the two tests occurred in four instances. One of these was due to the presence of nonprotein metabolite of radiopaque x-ray contrast medium for gallbladder visualization in the urine, which resulted in a false-positive reaction with the sulfosalicylic acid test but a correct-negative result with the tablet color test. Two other disagreements between the sulfosalicylic acid test and the tablet test occurred with urine samples which contained the metabolite of tolbutamide. This compound gives a false-positive reaction with sulfosalicylic acid but it does not react with the tablet. The fourth urine gave a strong reaction with the sulfosalicylic acid test and a negative reaction with the tablet. Study of this urine was limited by its small amount, and there was nothing in the patient's history to account for the discrepancy.

Table 2 presents the results of a study of 778 random urines from healthy subjects and hospital patients which were tested with the colorimetric strip test and with the sulfosalicylic acid test. Results were comparable to those obtained when sulfosalicylic acid and the tablet were studied. Six hundred and thirty urines were negative with both tests, whereas 77 urines were positive with both tests. In this experiment 5 urines gave results which disagreed. Here again one urine containing excreted metabolite of radiopaque medium for gallbladder visualization gave a false-positive with the sulfosalicylic acid precipi-

Table 2. Comparison of Colorimetric Strip Test with Sulfosalicylic Acid Test
Number of Urines Giving Designated Reaction

	Sulfosalicylic acid test		
	Negative	*Trace*	*Positive (1+ or more)*
Strip test—Negative	630	48	3
Strip test—Trace	18	–	–
		77	
Strip test—Positive (30 mg. or more)	2	–	–
Tests agree (Both positive or both negative)		90.9%	
Trace vs. Negative		8.5%	
Tests disagree (One positive other negative)		0.6%	

tation test and a correct-negative with the colorimetric strip test. Two urines containing excreted metabolite of tolbutamide gave false-positive precipitation tests with sulfosalicylic acid and correct-negative strip tests. Two urines that were correctly negative with the sulfosalicylic acid test gave 1+ reactions with the strip test—namely, a value of 30 mg. Both of these urines were highly buffered alkaline urines which will give false positive reactions with the strip test.

STUDIES ON URINES WITH ADDED PROTEIN

In order to compare the performance of the colorimetric strip test and the colorimetric tablet test, a series of experiments were carried out, using both experienced and inexperienced operators. To urines which were negative with both colorimetric tests, bovine albumin was added to give concentrations of 10, 30, 100, and 300 mg. per 100 ml. Each sample was given a code number and all of the tests were done as blinds, with each operator doing a single test on each sample. Sixteen hundred and eighty tests were done by 18 operators. Table 3 shows the distribution of results obtained with each concentration of protein with each of the colorimetric tests. It is obvious from these data that the two tests are similar.

COMPARISON WITH OTHER PROTEIN TESTS

In order to compare the performance of the colorimetric tablet test with the performance of common turbidity tests a series of unknown urine samples, many of which contained added bovine albumin, were submitted to both experienced and inexperienced operators. Figure 1 shows results obtained by two experienced operators with the

Table 3. Comparison of Tablet and Strip Colorimetric Tests for Proteinuria

Nature of sample	*Per Cent of Tests at Each Protein Level Showing Indicated Results*							
	Negative		*Trace*		*Small*		*Large*	
	Tablet	*Strip*	*Tablet*	*Strip*	*Tablet*	*Strip*	*Tablet*	*Strip*
No protein	100	97	0	2	0	1	0	0
10 mg. protein added per 100 ml.	22	15	75	76	3	9	0	0
30 mg. protein added per 100 ml.	0	0	1	0	99	100	0	0
100 mg. protein added per 100 ml.	0	0	0	0	92	100	8	0
300 mg. protein added per 100 ml.	0	0	0	0	2	10	98	90

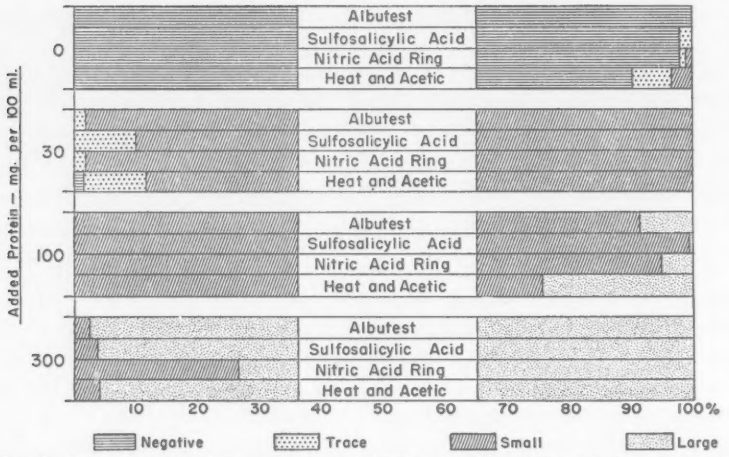

Fig. 1. Comparison of colorimetric tablet test and turbidity tests by experienced operators (3408 values).

sulfosalicylic acid test, the nitric acid ring test, and the heat and acetic acid test. A total of 3408 values were obtained over a period of several weeks, with a few tests being done each day with each method. It will be observed that the results obtained with the tablet test in this series were satisfactory, being slightly superior to those obtained with the other three tests.

A second series of comparative blind tests, using negative urines and samples which contained added bovine albumin, was carried out, using inexperienced operators who were unfamiliar with all of the tests. The procedure for each test was provided in written form to each operator. Results obtained in this series are shown in Fig. 2. It is evident that the precision with all 4 tests is considerably less than that obtained by the experienced operators. The inexperienced operators classified more of the negative urines as "trace" with the tablet test than with any of the other procedures. In urines with 10 mg. per 100 ml. of added protein the detection of protein was more readily achieved with the tablet test than with any of the three turbidimetric procedures. Significantly better detection of protein at the 20 mg. per 100 ml. level and at the 30 mg. per 100 ml. level was also obtained with the tablet test. Classification of 300 mg. per 100 ml. of protein as a large amount was more consistent with the tablet than with the turbidity tests.

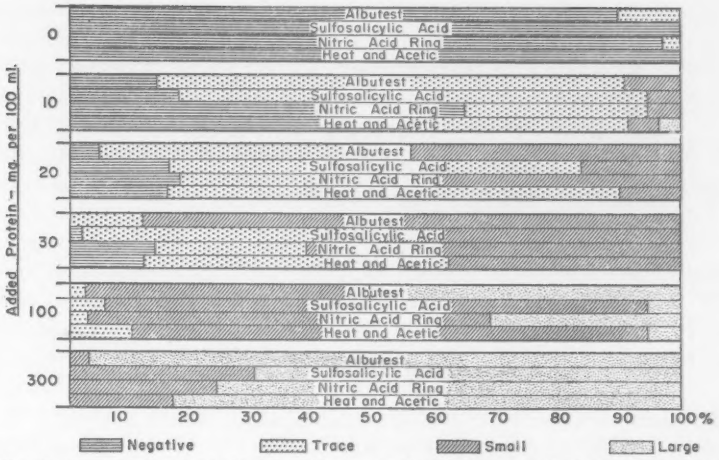

Fig. 2. Comparison of colorimetric tablet test and turbidity tests by inexperienced operators (1059 values).

A similar comparison of the colorimetric strip test with the turbidity tests has not been made on the magnitude of the experiments shown in Fig. 1 and Fig. 2. Data shown in Table 3, in which blind tests by experienced and inexperienced operators resulted in similar values with the tablet test and the strip test, suggest that the performance of the strip is comparable to the tablet.

SPECIFICITY

The specificity of the colorimetric tests is indicated by comparison with common turbidity tests in a large series of random urines. The specificity was also evaluated by investigating a number of substances which might be suspected of giving a possible false-positive reaction with either the tablet test or the strip test. The reactivity of several proteins and protein split products has also been studied. The results of specificity experiments are summarized in Table 4. In a series of over 2000 random urines from hospital patients and healthy subjects, no instance was found where the tablet test was clearly positive and the turbidity tests negative. This provides indirect evidence that urinary metabolites of commonly used drugs which can be expected to be present in random urines from hospital patients do not give false-positive reactions with the colorimetric tests. Two urines were encountered which were alkaline and strongly buffered, and

Table 4. SPECIFICITY OF COLORIMETRIC TESTS FOR PROTEINURIA

Reactive	*Nonreactive*
Albumins	Polyvinylpyrollidone
Globulins	X-ray contrast media
Hemoglobin	Urine preservatives
Bence Jones protein	Protein digests (very low activity)
Highly buffered alkaline urine (gives small reaction with strip)	Mucoprotein (very low activity)
	Amino acids
	Bilirubin
	Pyridium
	Inulin
	Dextran
	Metabolite of tolbutamide
	Metabolite of promazine
	Metabolite of chlorpromazine
	Metabolites of other common drugs
	Alkaline urine (does not give reaction with tablet)

these reacted with the strip but not with the tablet or turbidity tests. In comparison, metabolite of x-ray contrast medium for gallbladder visualization and tolbutamide metabolite gave strong false-positive reactions with the turbidity tests, but correct-negative results with the colorimetric tests.

In Table 4 are shown proteins which react with the tablet and the strip. On a weight basis, globulin is about one half as reactive as albumin. It is of interest that both hemoglobin and Bence Jones protein react with the colorimetric strips and tablets. In the case of enzymatic protein digests a small residual reactivity remains, but with either trypsin or pepsin proteolysis of albumin the activity after digestion is only 2 or 3 per cent of what it was before digestion. Similarly, purified urinary mucoprotein has a low activity which is only about 3 per cent as great as that of albumin. Calculations indicate that it would require a tenfold increase above normal urinary mucoprotein (3) in order for it to react with the colorimetric tablet or strip.

Since an acid-base indicator is present in both the strip and the tablet, it is important to establish the reactivity of the colorimetric tests with urines which are highly alkaline. Numerous trials have been made on this point, and in all instances the most alkaline urine that can be obtained from human subjects ingesting alkali and restricting their fluid intake shows no reactivity with the tablet unless

protein is present. Such urines containing no protein do show reactions with the strip comparable to that obtained with 30 mg. of protein in 100 ml. of urine. Unless a urine is highly buffered it will not give a positive reaction with the strip, even though it may have a *p*H as high as 8.

One urine sent to our laboratory gave an unusual reaction with the strip test. This urine, which was freshly voided, came from a patient with cystitis and urinary retention, had a *p*H of approximately 9.5, and effervesced when tested with sulfosalicylic acid or with either of the colorimetric tests. The specimen contained protein, but because of its high alkalinity did not precipitate with sulfosalicylic acid. The color reaction of the strip was exaggerated by the alkalinity of the sample and the effervescence with the tablet distorted its usual appearance.

Several polymers such as Dextran, polyvinylpyrollidone and inulin were all studied by addition to urine, and polymer added to each sample gave completely negative results with the tablet and the strip. Certain dyes or pigments which appear in the urine such as Pyridium, Serenium, metabolite of chlorpromazine, promazine, and methylene blue may give confusing results with turbidity tests for protein. None of these gave any difficulty with either the strip or the tablet.

STUDIES WITH TURBID URINES

Twelve per cent of a series of urine samples collected from hospital patients showed some turbidity at the time they were voided. When the urines were allowed to stand at room temperature for approximately 6 hours, the number of turbid specimens increased to 20 per cent. Refrigeration at 40° F. resulted in 38 per cent of the specimens showing turbidity. There was not a significant change in the number of turbid samples when they were returned to room temperature, but there was a decrease in the degree of turbidity in many of the samples. Where facilities for centrifuging or filtering urines are at hand, turbidity can be overcome in the majority of instances, but in some cases it is still a problem. One of the big advantages of the colorimetric tests is the fact that they can be used on turbid urines to obtain correct results.

A series of turbid urines were collected and to aliquots of these, bovine albumin was added. Experienced operators carried out approximately 200 blind tests on these samples with the sulfosalicylic acid test, and a comparable number with the colorimetric tablet test.

In approximately 50 per cent of the tests, satisfactory results were obtained with the sulfosalicylic acid test, whereas satisfactory results were obtained in 99 per cent of the tests with the tablet test. A comparable high level of performance has been seen with the strip test on turbid urines under the same conditions.

SENSITIVITY

Information on the sensitivity of the colorimetric tests can be obtained from Figs. 1 and 2 and Table 3. Inexperienced operators in blind testing reported positive results with the tablet test in 87 per cent of urine specimens containing 10 mg. of bovine albumin in 100 ml. of urine. In the same urines the operators were able to detect protein with the sulfosalicylic acid test in 84 per cent of the cases, whereas with the nitric acid ring test, and heat and acetic acid test less than half such specimens were recognized as containing protein. Similarly, better results were obtained with the colorimetric tests than with the turbidity tests in detecting 20 and 30 mg. of added albumin per 100 ml. Table 3 indicates that the sensitivity of the strip and tablet are comparable in detecting 10 mg. of added albumin.

Data presented in Tables 1 and 2 indicate that a greater number of trace reactions in random urines can be expected with the sulfosalicylic acid test than with either of the colorimetric tests. Such data may be interpreted in either one of two ways. It can be considered that the sulfosalicylic acid test is more sensitive than the colorimetric tests and, therefore, capable of detecting smaller quantities of protein. A second interpretation which seems equally valid is that the substance or substances naturally excreted in these urines is not albumin, but may be some other protein or even a nonprotein component as pointed out by McMillin (4) and Bergstrom (5). Rigas and Heller (6) have shown that healthy subjects excrete in their urine, per 24 hours, approximately 40 mg. of protein, about two-thirds of which is globulin. Since these two proteins do not behave the same toward either the colorimetric or sulfosalicylic acid tests (7), it is possible for one test to respond more readily than the other to urines containing different ratios of albumin and globulin. The nature of materials responsible for producing turbidity with sulfosalicylic acid, but no color with the tablet or strip, is a subject for further study.

A third approach in attempting to define sensitivity has been made by adding extremely small amounts of albumin to urines that are completely negative to the turbidity and colorimetric tests. When such a

negative sample is tested along with an aliquot of the same sample containing added protein, it is possible to distinguish by either turbidity or colorimetric tests amounts of added albumin as small as 1 to 4 mg. per 100 ml. of urine. This observation is of theoretical interest, but does not give an indication of the practical sensitivity of any of these tests.

TIME OF PERFORMANCE

Persons familiar with each of the tests carried out a series of 10 tests with each of the methods. A second person accurately timed each series, with time for handling and cleaning glassware being included. Average performance times calculated on a single test basis were as follows: heat and acetic acid test—70 seconds; nitric acid ring test—40 seconds; sulfosalicylic acid test—28 seconds; colorimetric tablet test—14 seconds; and colorimetric strip test—less than 10 seconds.

SUMMARY

A color reaction of protein has been formulated into two simple colorimetric tests for protein in urine; one is a tablet test, consisting of a salicylate buffer and bromphenol blue, and the other is a strip test, consisting of a paper strip impregnated with a citrate buffer and tetrabromphenol blue. The basic principle in both tests is that of "protein error of indicators." Experiments with approximately 5000 urines, taken from both hospital patients and normal subjects, reveal that these tests are simple, rapid, accurate, sensitive, specific, and capable of being used with turbid urines.

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A New Colorimetric Method for the Determination of Isoniazid in Biologic Fluids

Vincenzo Scardi and Vincenzo Bonavita

In a previous paper (1) a rapid colorimetric method for the determination of isoniazid (INH) in blood serum or plasma was described. The method was based on the reaction between isoniazid and sodium pentacyanoamineferroate, $Na_3[Fe(CN)_5NH_3]$, and on the subsequent colorimetric evaluation of the yellowish chromogen at 430 mμ.

The determination, however, of INH in cerebrospinal fluid (CSF) by this method was not possible because the deproteinization procedure used for serum or plasma was unsuitable for the relatively low protein content of CSF, even in pathologic conditions requiring INH estimation.

In the attempt to overcome this difficulty we succeeded in finding a new satisfactory deproteinization procedure for CSF; moreover, it is also suitable for serum or plasma and represents a further simplification in respect to the original method.

It is the purpose of the present communication to describe the new rapid method for the INH determination.

REAGENTS

1. *Isonicotinic acid hydrazide,* 0.1 per cent stock solution. This solution must be stored in a refrigerator and discarded after a week.
2. *Isonicotinic acid hydrazide,* standard solution (100 μg./ml.), to be prepared from the stock solution just before use.
3. *Metaphosphoric acid,* 20 per cent.

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Received for publication August 4, 1957.

4. *Diammonium phosphate,* $(NH_4)_2HPO_4$, 16.5 per cent.

5. *Sodium pentacyanoamineferroate,* 0.2 per cent in 0.02N NH_4OH.

Since this substance is not commercially available it must be prepared in the laboratory. Ten grams of finely powdered sodium nitroprusside are treated in a flask with 32 ml. of concentrated ammonium hydroxide overnight, at refrigerator temperature. Absolute ethanol is added to the mixture and a yellow precipitate is obtained. The precipitate is washed with absolute ethanol and anhydrous ether until dry. The resulting sodium pentacyanoamineferroate must be stored in a desiccator. The 0.2% solution of sodium pentacyanoamineferroate in 0.02N NH_4OH is stable at room temperature.

ANALYTIC PROCEDURE

The steps in the analytic procedure are identical for serum, plasma, and cerebrospinal fluid, except for the use of different amounts of the sample to be analyzed.

One milliliter of fresh serum is added to 2 ml. of distilled water in a centrifuge tube and is well agitated. One ml. of 20% metaphosphoric acid is added, and the tube is allowed to stand at room temperature for about 10 minutes before centrifuging for the same period at 4000 r.p.m. In the case of CSF 2 ml. of fluid are added to 1 ml. of water in the centrifuge tube. The protein precipitate packs well in the bottom of the centrifuge tube and 2 ml. of clear supernatant are easily pipeted off and transferred to a colorimetric tube. Five tenths milliliters of diammonium phosphate and 0.5 ml. of sodium pentacyanoamineferroate are added, shaking well after each addition.

A yellowish color develops maximally within 5-10 minutes and is stable at least for 20 minutes with only a very slight tendency to decrease in intensity. The intensity of the color is measured in a Klett-Summerson colorimeter with a No. 42 (blue) filter against a blank prepared by substituting water for serum or CSF samples. Any other colorimeter can be used, and the final volume can be modified without varying the relative proportion of each reagent.

There is a straight line relationship between the colorimetric readings and INH concentration (Fig. 1).

ACCURACY AND SPECIFICITY

Recovery experiments in which INH was added to serum and to CSF are summarized in Table 1. Standard deviation calculated on 10 determinations of the same sample containing 20 μg./ml. is $\pm$ 0.29 μg.

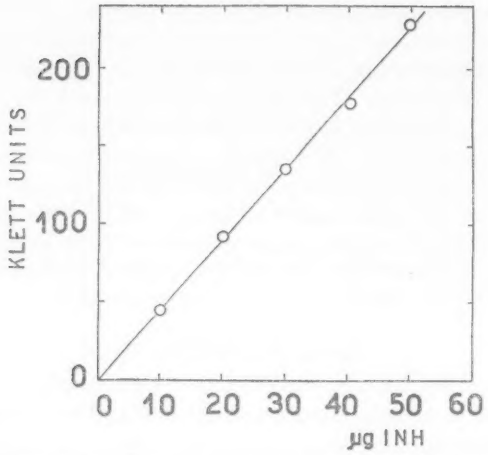

Fig. 1. Calibration curve of the yellow compound produced in the reaction between INH and $Na_3[Fe(Cn)_5NH_3]$, showing linear relationship with the blue No. 42 filter (420-440 mμ).

$$\text{Klett colorimeter units} = \frac{\text{Absorbance}}{2} \times 1000.$$

Table 1. Recovery of INH Added to 1 ml. Serum and 2 ml. CSF

Biologic fluid	*INH added (μg.)*	*INH found (μg.)*	*Recovery (%)*
Serum	5	5	100.0
	10	10.2	102.0
	20	19.9	99.5
CSF	5	5	100.0
	10	10	100.0
	25	24.8	99.2
	50	50	100.0

Both serum and CSF actually do not react with $Na_3[Fe(CN)_5NH_3]$ in absence of INH, although a more accurate spectrophotometric analysis of the serum or CSF filtrate treated with $Na_3[Fe(CN)_5NH_3]$ show a very slight absorption, which is practically negligible in the 420-440 mμ region.

In respect of other methods for determination of isoniazid in blood serum and CSF, the present method is superior in regard to the amount of biologic fluid required (2), the rapidity, and the simplicity. The method is very specific for isoniazid in as much as $Na_3[Fe(CN)_5$-

NH_3] does not react with other hydrazides to any appreciable extent. Nicotinamide, however, represents the unique interfering substance of biologic interest so far detected (1, 3), but the absorption maximum of the chromogen compound does not coincide with that of the INH and the extinction coefficient ratio, in the wavelength range chosen for colorimetric readings, is 1:5.

SUMMARY

The authors describe a new colorimetric method for the determination of isoniazid in blood serum and cerebrospinal fluid.

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A Toxicologic Service in a General Hospital

Sidney Nobel

THE PURPOSE OF THIS ARTICLE is to present useful information and suggestions that have been found of practical value in a general hospital in dealing with problems arising from accidental, suicidal, and industrial intoxication. This material is based on background experience gained in the laboratory of Dr. A. O. Gettler, Chief Toxicologist to the Medical Examiner of the City of New York, and in the establishment of a toxicologic service in a community hospital. As seen below, the clinical chemist can contribute materially to the cooperative effort required in the proper handling of a toxicologic problem.

ORIENTATION OF THE EMERGENCY ROOM

A good working relationship between the emergency room and the laboratory is critical to the efficient handling of acute poisoning cases. A series of informal discussions between the two departments concerning the contributions that each can make in poisoning cases has made for better liaison. The following points were covered in the discussions.

1. *Specimen Collecting.* The first gastric wash should be submitted for examination because it contains the highest concentration of toxic substance. By the third gastric wash, a toxic substance may be so dilute as to make analysis impossible. Blood specimens, when required, should be drawn as early as feasible. In carbon monoxide

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Portions of this paper were presented in an exhibit before the International Congress of Clinical Chemistry, September 9-14, 1956, New York, N. Y.

The author is indebted to Dr. A. O. Gettler and members of his staff, Dr. H. Schwartz, Mr. D. Posnick, and Mr. S. Schenkel for their valuable suggestions and to Dr. M. Rush, Director-Pathologist, for his aid in the preparation of this manuscript.

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Received for publication June 27, 1957.

cases, for example, a specimen drawn after prolonged oxygen therapy may give misleading results. Vomitus should be regarded as a valuable specimen for analysis because it may contain the toxic substance in relatively concentrated form and thus facilitate the analytic problem.

2. *Clue Collecting.* There is an almost infinite number of poisonous substances with only a limited amount of biologic material and time available for analysis. Thus the emergency room is depended on for information that will help direct the analysis toward the rapid identification of the toxic substance.

Although the treatment of many poisons is symptomatic, it is important to establish a diagnosis as quickly as possible. For example, a misleading state of depression may be due to exhaustion following convulsive seizures. The following types of information are generally helpful to the analyst: the circumstances under which the patient was found, the clinical picture, the occupational history, and familial knowledge of the patient's preference for specific drugs. All natural or commercial products brought in with the patient should also be submitted to the laboratory. One must also consider the possibility that some of the cases considered at first glance as an organic disease may, in fact, be due to some toxic substance. A classic example of this is the mistaken diagnosis of a carbon tetrachloride form of hepatitis as a viral hepatitis.

3. *Antidotes and Therapy.* Copies of two books (1, 2) containing information on the diagnosis and treatment of poisoning were presented to the emergency room. The toxicity of some of the antidotes was pointed out and extreme caution advised in their use. A check was made of the antidotes and equipment on hand using the lists in chapter 3 of Von Oettigen's book (2) as a guide. The missing equipment was procured and the required antidotes placed in a special cabinet with written warnings concerning the use of the more dangerous ones.

4. *Diagnostic Aid.* An odor reference shelf was placed in the emergency room to aid in the rapid identification of those poisons with characteristic odors. Some of the chemicals on this shelf are carbon tetrachloride, cyanide, elemental phosphorus, kerosene, nitrobenzene, paraldehyde, petroleum ether, phenol, and sodium hypochlorite. It was emphasized that the absence of odor does not elminate the possibility of contact with any of these poisons.

GENERAL RULES OF PROCEDURE IN THE LABORATORY

1. The analyst must be aware of extreme personal danger in dealing with unknown compounds. For example, an unmarked paper bag filled with grayish granules was found near a dead man. The granules were identified as calcium cyanide, a product used in commercial exterminating, which liberates hydrogen cyanide even in neutral aqueous solution. Autopsy confirmed cyanide as the cause of death.

2. The analyst must be alert to the possibility that a toxic compound found near the patient or even in the gastric contents may not be primarily responsible for the observed clinical picture. There are numerous cases, especially among suicides, where more than one poison is involved. Sometimes this occurs because the first poison is relatively slow acting, thus giving the victim enough time to hunt up another toxic substance.

3. By preparing a master chart of many of the capsules and pills manufactured by pharmaceutical houses, the analyst may simplify the identification of proprietary drugs. Each pill or capsule is placed in a test tube labeled with the trade name and chemical composition. Fortunately, the capsules are made in a wide range of color combinations, shapes, and sizes. It is thus possible to identify an unknown capsule in a matter of seconds if it can be matched with one in the collection. A confirmatory chemical test should always be run because of the possibility of duplication of shape and color by different drug houses.

4. The analyst may have to rely on many sources of information in obtaining a lead on the chemical composition of commercial and natural products. Several books have been found to be especially helpful (3, 4, 5). A careful reading of the label may also give valuable information. The nearest Poison Control Center (5) may have the desired information in its files. As a final recourse one may contact the manufacturer directly.

ANALYTIC PREPARATIONS

Although the total number of poisons is enormous, only about 19 compounds or their analogs are involved in the majority of cases. These are antimony, arsenic, barbiturates, carbon monoxide, chlorinated hydrocarbons, cyanide, ethyl alcohol, fluoride, formaldehyde, iodine, lead, mercury, methyl alcohol, morphine, paraldehyde, phenols, phosphorus, salicylate, and strychnine. Prior preparation of methods, apparatus, and reagents to detect and to determine quan-

titatively these poisons in biologic fluids is to be considered as an initial step in adequately handling most cases of acute and chronic toxicity encountered by the laboratory.

Simple methods may sometimes be selected (6, 7, 8) which are not a burden to a laboratory with limited space and equipment. More than half the poisons mentioned can be detected with the aid of glass flanges, Conway dishes, and a copper coil. For example, the aeration of 1 μg. of cyanide (9) through an alkali-ferrous sulfate-treated disc of filter paper supported by two glass flanges will produce a blue color in 5 minutes. There are similar methods for the determination of carbon monoxide (10) using a palladious chloride-impregnated filter-paper disc, and for elemental phosphorus (11) using a silver nitrate-treated disc. These methods may be used quantitatively by comparing the color produced with the colors of standards run under identical conditions.

Conway methods have been developed for the major toxic volatile compounds (12). Although they usually require more time than the aeration methods to produce an analytic result, their simplicity is a compensating factor. This liability has been overcome in a blood-alcohol method (13) by incubating the Conway dish at 90° for 20 minutes.

The utility of the Reinsch test (14) has been increased by a scheme of analysis able to detect amounts of mercury, bismuth, arsenic, antimony, selenium, and tellurium that are invisible on the copper collector.

The frequency of encounter with salicytates and barbiturates is responsible for methods (15, 16) of their analysis being generally available in the clinical laboratory. The determination of other nonvolatile poisons may require more complex methods (17, 18, 19). Newer technics involving the use of filter-paper chromatography, ion exchange resins, and both ultraviolet and infrared absorption have been applied to the determination of nonvolatile poisons (20).

Sometimes a method is too recent to have gained access to published volumes. This is found to be especially true in attempts to analyze some of the newer pharmaceutical preparations. One may contact the pharmaceutical firm directly for aid or consult the current literature. It has occurred that a firm was unable to suggest an adequate method for the analysis of their product in a biologic system, yet a method was located in *Chemical Abstracts*.

PARTICIPATION IN PUBLIC HEALTH PROGRAMS

The hospital may help attack the problem of accidental poisoning in the home at its source by participating in a variety of public-health education programs. This may range from talks to parent-teacher associations or other interested groups to the preparation of exhibits at community fairs. At a recent local fair an exhibit was prepared with the theme, "Poisoning in the Home." The central poster was of a child reaching into a medicine chest with the caption, "Don't let your child become a statistic!" Figures for injury and death due to accidental poisoning in children, lists of common dangerous household items, and a method for the conversion of common kitchen items into universal antidotes were exhibited. In addition, there were several case histories of child poisoning with a demonstration of the chemical methods involved. In this area, efforts in the direction of public awareness are as important as technical preparation to handle the most complex toxicologic problem.

SUMMARY

Information and suggestions found of value in a general hospital in dealing with toxicologic problems are presented. Relevant problems such as good liaison between the emergency room and the laboratory, sources of information on product composition, the preparation of an odor reference shelf, and selection of methods to help meet the majority of situations encountered have been discussed. The importance of participation in public-health education programs has also been indicated.

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A Semimicro Technic for the Measurement of *N,N*-Dimethyl-*P*-Phenylenediamine (DPP) Oxidase in Blood

Leontine Goldschmidt

THE APPEARANCE of Akerfeldt's paper (1) on oxidation of DPP by serum from patients with mental disease was preceded by press reports of his findings several months in advance (2). Because of their possible significance [recently reviewed by Abood (3)], press reports inspired attempts to confirm the findings in many laboratories, including our own, prior to their scientific publication. In the course of our studies, certain technical difficulties were encountered and overcome. The semimicro method developed by us permits rapid serial determinations to be made, and is therefore economical of material and of time.

DEVELOPMENTAL STUDIES

Initial work with the reaction differed in technic from that of Akerfeldt in employing (a) DPP base and (b) DPP hydrochloride instead of dihydrochloride. The base was soon considered impractical, as it is difficult to dissolve quantitatively and tends to form precipitates.

DPP HCL was found to be more readily tractable than base, since it will dissolve easily and does not precipitate. Upon addition of an equal volume of plasma or serum the rate of oxidation of this solution was found to be linear and to have a slope apparently characteristic for the individual, but difficult to express for comparative purposes (Fig. 1).

From the Creedmoor Institute for Psychobiologic Studies, Queens Village, N. Y.

The author acknowledges with pleasure the assistance of John R. Whittier, M.D. in preparation of this paper.

Received for publication July 27, 1957.

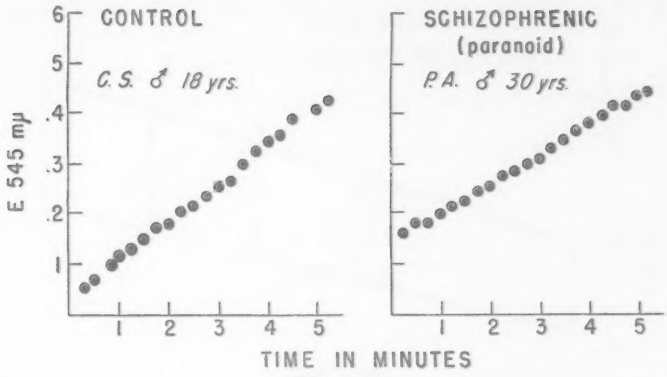

Figure 1.

Furthermore, allowance had to be made for the autoxidation of DPP in aqueous solution before interpretation of the oxidation of the solution by serum or plasma could be ventured.

The light absorption curve of a solution of DPP base in 0.85% NaCl showed a slight plateau at 540-550 mμ. The optical densities of a series of solutions of DPP base ranging from 0.5 per cent to 0.1 per cent was measured at 545 mμ and found to be linear.

Initial experiments were performed by mixing 1 ml. of a 0.2 per cent solution of recrystallized DPP HCl in isotonic buffer *p*H 7.0 (4) with 1 ml. of serum or plasma, and by reading after exactly 5 minutes the optical density of this mixture at 545 mμ against a blank made up of a mixture of 1 ml. serum or plasma and 1 ml. of buffer. The autoxidation of the DPP HCl solution was determined simultaneously against a blank of buffer, and accounted for by expressing the oxidation of the serum or plasma containing mixture in per cent of increase of optical density over dye solution, or per cent I.

$$\%I = 100\ \frac{[D\ (0.1\text{ per cent Dye} + \text{Plasma} + \text{Buffer}) - D\ (0.1\text{ per cent Dye} + \text{Buffer})]}{D\ (0.1\%\ \text{Dye} + \text{Buffer})}$$

D = Density at 545 mμ after 5 minutes

Thus, if the optical density of the autoxidized dye solution were 0.36 and the optical density of the plasma-dye mixture 0.54, I would be 50 per cent (Fig. 2).

The per cent I of our control specimens (mostly from employes) rarely exceeded 25 per cent, whereas the per cent I values of the ma-

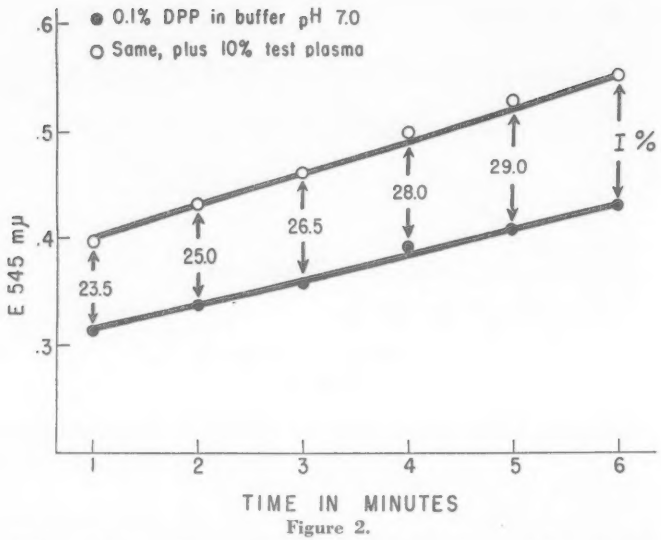

Figure 2.

jority of patient specimens (from hospitalized mental patients) did exceed this value, frequently to a significant degree.

We discovered, in line with other investigators (5a), the great influence that *p*H has on this reaction. An optimum was established at *p*H 7.0, as the reaction tended to proceed at a very fast rate in acid solution and precipitates of DPP base were encountered in alkaline medium.

The comparatively elaborate procedure of *p*H control which was necessitated by the high proportion of serum or plasma used in our initial experiments—such as freeing the sample of CO_2—constituted one of our primary incentives for the development of a semi-micro technic.

A further important advantage of such a method was seen in the lightening of the specimens' natural color by high dilutions in buffer. We found that we could, for all practical purposes, dispense with individual plasma and serum blanks, and thus proceed with serial determinations.

With others (5b), we found that hemolysis had to be avoided, because it contributed to high readings. Plasma of slightly heparinized blood was used for most of our determinations, since it was less liable to hemolysis than clotted blood. Comparative readings on

plasma and serum samples from the same subject showed no differences.

MATERIALS AND REAGENTS

Screw-cap tubes, 10 ml., containing a small drop of dried sodium heparinate.

N, N-dimethyl-*p*-phenylenediamine HCL (DPP HCL) Eastman, recrystallized from methanol.

Isotonic NaCl-phosphate buffer, *p*H 7.0 This buffer is made by mixing 75 ml. of 1.2 Gm/100 ml. NaCl with 25 ml. of a mixed buffer, composed of 72 ml. of M/15 Na_2HPO_4 and 28 ml. of M/15 KH_2PO_4(4).

Coleman cuvets 6-308 (3×0.5 inches)

Micro pipets, Folin graduated at 0.2 ml.

PROCEDURE

Venous blood, drawn in the customary manner, is dispensed into heparinized tubes. The samples are inverted twice very gently, allowed to sediment for about an hour and centrifuged at 2000-3000 rpm. The plasma is separated and centrifuged. Determinations are made as soon as possible, although immediacy is not essential.

Two-tenths of a milliliter of each plasma specimen is added to 1 ml. of isotonic buffer in a Coleman cuvet. A 0.2 per cent solution of DPP in isotonic buffer is prepared and left to "mature" until it shows an optical density of 0.5 to 0.6 when read at 545 mμ in a Coleman cuvet. This process usually takes 15 to 25 minutes.

At this point 1 ml. of the dye is dispensed into each plasma sample. Stop-watch timing begins simultaneously with addition of the dye to the first tube.

As precise timing is essential, it is recommended that each series should not contain more than 10 tubes, in order to make it possible to dispense the dye solution from a graduated pipet without refilling. This process should take no more than about 30 seconds. Each sample should be mixed by shaking after the stage of dye addition is completed for the entire series.

The first and the last tube of each series should consist of "dye controls" composed of 1.2 ml. of buffer and 1 ml. of dye solution. Furthermore, each series should contain at least two control specimens from normal individuals. The blank for the entire series consists of buffer.

As it is difficult to time all of the readings accurately, the reaction is arrested after exactly five minutes by adding to each tube from a

Wintrobe pipet 1 large drop of a 0.2 per cent NaCN solution in isotonic phosphate buffer. The drop of cyanide is allowed to fall into the test tube instead of running down its wall. With some practice it is possible to do this at the same rate at which the dye was added. In this procedure, too, it was found necessary to complete the series before mixing the individual tubes. The cyanide solution will arrest the reaction for about a minute. At this point the entire series is read. Per cent I values are calculated by the formula:

$$\frac{100\ [D\ (0.1\ \text{per cent Dye} + \text{Plasma} + \text{Buffer}) - D\ (0.1\ \text{per cent Dye} + \text{Buffer})]}{D\ (0.1\ \text{per cent Dye} + \text{Buffer})}$$

which was developed at the beginning of this paper.

The dye control tubes at the beginning and at the end of the series should show virtually identical readings if the reaction is properly performed. The general range of the dye control readings is from optical density 0.32 to 0.42 with an average of 0.37.

It is recommended that optical density of each sample in the entire series should be "preread" between 4 and 5 minutes after addition of the dye, to provide additional control, although the final readings of optical density are obtained after addition of the cyanide solution. High optical density of 0.5 and over may require 2 drops of cyanide solution. The proper amount of inhibitor, which must be kept at a minimum, will be learned by short practice. It is, furthermore, recommended that fasting plasma be used on account of its comparative clearness.

DISCUSSION

Although the results obtained by this method will be discussed in later papers it can be stated that significant differences were obtained between groups of hospitalized psychotics and control individuals of corresponding age.

The procedure described in this paper provides an index of DPP oxidase activity, although it may not be called "quantitative," as it is basically an enzymatic reaction.

The advantages of our method over methods already reported are: (a) a comparatively small amount of serum or plasma is needed; (b) it permits of serial, rapid, and reproducible determinations; and (c) the results remain constant through several days and the necessity for immediate determination is thereby obviated.

SUMMARY

A semimicro method for the evaluation of DPP oxidase activity in blood serum and plasma has been reported and its advantages have been described.

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the Clinical Chemist

PRESIDENTIAL ADDRESS

Joseph I. Routh

A MAJOR FUNCTION of the annual presidential address is to acquaint the members with the achievements of the Association during the year just past. In addition to the very successful International Congress of Clinical Chemistry held in this city a year ago, an accounting might be made of other activities:

Our journal, CLINICAL CHEMISTRY, continues its healthy growth, our participation in symposia and programs of other societies is increasing, and our representation on the American Board of Clinical Chemists is most satisfactory.

Committee activity during the year has been encouraging. As a result we may look forward to the publication of the second volume of *Standard Methods,* which should be off the press about the first of the year.

Liaison with associated societies, such as the American Chemical Society, the American Society of Biological Chemists, and the American Association for the Advancement of Science, continues to improve.

For those of you interested in a new position in clinical chemistry the employment service of the Association regularly received and dispatched notices of openings in the field. Approximately thirty-five openings, at various levels, have been brought to the attention of interested members. In addition to bringing these openings to the attention of interested members, consultations with prospective employers has resulted in an improvement in the salaries offered to clinical chemists.

With this brief backward look over the year's activities we may now turn our attention to the present and the future.

In a little more than another year our Association will have reached its first decade of existence. In the early years growth has been very rapid and marked by the appearance of a volume of standard methods, a successful journal, and by recognition from many associated societies. We have now reached the stage of young manhood where

Presented at the Ninth Annual Meeting Dinner, New York, N. Y., September 12, 1957.

we must prepare to meet the responsibilities of adult life. The success of the venture has obviously been due to the hard work and intense idealism of the originators of the Association, who have guided its policy through the formative years. The mechanisms by which the affairs of the Association have been governed served admirably when the group was smaller and relatively compact. Our membership has increased and spread throughout the country in recent years. At present there are nine local sections representing clinical chemists in at least sixteen states. A tenth has petitioned for membership and a charter has been granted. There is every reason to believe that additional sections will be formed as the membership expands. It is no longer wise or sufficient to depend on the handful of volunteers who have borne the burden of the Association's affairs, nor does the continuance of such an arrangement permit a widespread feeling of awareness and participation among the membership in general.

The most successful societies employ some system of broad representation in their governing body to promote a ready exchange of ideas and opinions between it and the membership, or vice versa. We have now reached the stage in our development where it is essential that we adopt a procedure based on similar philosophic principles. The formation of local sections is evidence of interest and activity of clinical chemists in a particular geographic area. To encourage section activity and responsibility and to foster the formation of additional sections the government of the Association should be placed as far as possible in the hands of qualified representatives of these local sections.

The governing board of the Association, be it called Executive Committee, Council, House of Delegates, or otherwise, should include representatives chosen by the local sections who are responsible not only to the Association but also to the section from which they were elected. Such a system would broaden national responsibility and would provide the essential channels of information from the governing body to each local section. Since the election of the representatives would be carried out in the local sections where more information would be available on each candidate, the national body should consist of well-qualified individuals.

The problem has been under intensive study for several years, and in culmination the executive committee has formulated several constitutional changes. The fundamental feature of the proposed changes would involve the size and scope of the National Executive Commit-

tee by including as full voting members representatives elected by each local section. The proposed changes will impose a great responsibility on each local section. The intent of the proposal will be served only if each section selects a responsible member who will not promote factionalism, who will have the interest of the organization and of the science at heart, and who will be faithful in his liaison between the section members and the national body. The membership will shortly receive an exact statement of the proposed changes. We urge that this be made the subject of careful study by each member. We further urge that every member shall express his opinion by voting on the proposed changes. If the proposal is adopted each section should give careful and detailed attention to the choice of their representative. Our mutual interests can best be served only by the very best that each section can afford.

MINUTES OF THE MEETING OF THE A A C C EXECUTIVE COMMITTEE

The National Executive of the American Association of Clinical Chemists met in New York City on September 11, 1957. Those present were JOSEPH I. ROUTH, (President), OLIVER H. GAEBLER (Vice-President), MAX M. FRIED'AN (National Secretary), LOUIS B. DOTTI (National Treasurer), FERRIN B. MORELAND, DAVID SELIGSON, ROBERT L. DRYER (Alternate for ELIZABETH POMERENE), JOSEPH T. GAST (Alternate for RICHARD J. HENRY), JOHN G. REINHOLD (Alternate for ROBERT M. HILL), and the following by invitation; HAROLD D. APPLETON, MONROE E. FREEMAN, HUGH J. MCDONALD, ALBERT E. SOBEL, HARRY SOBOTKA, and WARREN M. SPERRY.

Committee Reports

Gaebler reported on the International Congress on Clinical Chemistry held in Stockholm, Sweden, in August, 1957. Many nations were represented by clinical chemists and the program was well received.

Dotti reported for the Committee on Membership. At the meeting date there were 721 members in good standing. Additional applicants were being processed.

Appleton reported for the Board of Editors of CLINICAL CHEMISTRY. The journal circulation is steadily increasing, and the royalty for the year 1956 amounted to $1,972.35. The supplement containing the symposia papers of the International Congress of 1956 consists of 314 pages of text and was published with the August, 1957, issue of the journal. The cost to the Association of this supplement will be about $5700.00 and to be considered as an expense of the Congress.

Seligson reported for the Committee on Methods and Standards. The Association has accepted an invitation from the National Research Council to participate in the program

of standardization of the cyanmethemoglobin method. Also Volume 2 of *Standard Methods* to be published by the Academic Press was now in galley proof and Volume 3 has been partly allocated to various authors.

Reinhold reported for the Nominating Committee. The slate of officers for the 1958-59 term prepared by this committee will be announced.

Dotti submitted the Treasurer's report which follows:

TREASURER'S REPORT
July 1, 1956 to June 30, 1957

Income	
Membership dues	$ 8,385.22
Royalties, Academic Press	101.36
Royalties, Paul B. Hoeber, Inc.	1,972.35
International Congress, 1956	3,832.33
Directory	50.00
Membership certificates	8.00
Bank interest	146.27
TOTAL	$14,495.53
Expenses	
Subscriptions, CLINICAL CHEMISTRY	$ 4,362.00
Editors, CLINICAL CHEMISTRY	324.61
National Secretary, Directory	891.39
National Treasurer	161.60
Membership Committee	95.20
Methods Committee	250.00
AAAS Meetings	43.39
Section allotments	471.30
International Congress, 1956	4,919.50
Return on subscriptions	28.70
Ernst Bischoff Award	64.63
TOTAL	$11,612.32
Total income	$14,495.53
Total expenses	11,612.32
Balance	$ 2,883.21
Bank balance, June 30, 1956	7,698.14
Bank balance, June 30, 1957	$10,581.35

Membership Requirements

At the request of a local section a discussion was undertaken concerning the evaluation of membership requirements, especially at the associate level. It was the consensus of the Executive Committee that the present requirements were adequate and no change was contemplated at this time. A motion was made, seconded, and passed that clinical chemists engaged in the field of veterinary medicine and who meet the Association requirements be admitted to membership in the Association.

A motion was made, seconded, and passed that an Emeritus Member status be created. Members who fully retire with continuous membership since 1950, or of ten years duration, may apply for emeritus status with full membership privileges without the payment of dues and to include the option of a journal subscription at the allotment rate which at this time is $6.00 per year.

Criticisms concerning the Ernst Bischoff Award were directed at the membership. There was a lack of cooperation in the proposal of nominations. It was recommended that the officers specifically request each local section to make nominations for the Award and the policy be established

that past nominators be canvassed as to their desire to renominate their candidate.

A group of members residing in New York State has petitioned for a local section charter. It was moved, seconded, and passed that since the petitioners have met the constitutional requirements for a local section that such a charter be granted to the Western New York Section and the exact areas to be established by the section.

The contract with Paul B. Hoeber, Inc., for publication of CLINICAL CHEMISTRY will expire on December 31, 1959. A preliminary discussion of the contract renewal contained the following recommendations:

1. The contract be drawn up with legal advice.
2. The present allotment by the publisher for line cuts and special composition of $40.00 for each journal article be revised to an allotment per issue basis rather than per article.
3. The Journal to be published on a monthly basis as soon as possible.

It was moved, seconded, and passed that the sum of $250.00 be allocated to the editors to cover extra charges for line cuts and special composition costs of journal articles.

A committee was appointed to negotiate the contract renewal, the committee to consist of HAROLD D. APPLETON, JOSEPH H. GAST, and WARREN M. SPERRY.

It was moved, seconded, and passed that the National Secretary be instructed to request from the International Federation of Clinical Chemistry approval for an International Congress on Clinical Chemistry to be held in North America during 1962, the exact time and site to be determined subsequent to such approval.

The Committee on Constitution introduced a preliminary draft for the revision of the present constitution. The final draft will be presented to the membership for a mail ballot.

It was moved, seconded, and passed that the next Stated Annual Meetings be held at the time and place of the meetings of the Federated Societies in April, 1958 at Philadelphia.

It was moved, seconded, and passed that the Tenth Annual Meetings of the Association be held in the late summer or early fall of 1958 at a university campus that would be host to such a meeting. Iowa City was considered a preferred location if arrangements could be made there.

It was moved, seconded, and passed that the Executive Committee recommend to the membership that dues for 1958 remain at $12.50 for members and $9.00 for associate members. (This was subsequently voted at the membership meeting on September 12, 1957.)

MAX M. FRIEDMAN
National Secretary

STATED ANNUAL MEETING

The Stated Annual Meeting was held in New York City on September 12, 1957.

The National Secretary reviewed the transactions of the Executive Committee who had met on the previ-

ous day. The Executive Committee recommended to the membership that the dues for 1958 remain at $12.50 for members and $9.00 for associate members. A motion was made, seconded, and passed that the dues be kept at the above figures.

The chairman of the Board of Editors reported on the progress of CLINICAL CHEMISTRY and exhibited the supplement containing the symposium papers of the International Congress in 1956.

The chairman of the Committee on Methods announced that volume 2 of "Standard Methods" published for the Association by the Academic Press was now in galley proof. Volume 3 was in preparation.

The main portion of the meeting was devoted to a discussion of expansion of the Journal and ways and means to further improve it. Special attention was given to the need for more clarity in the description of methods as published in the Journal.

ASSOCIATE MEMBER DUES

The following item is from the minutes of the Executive Committee meeting held at Minneapolis, Minn., on September 14, 1955, and published in CLINICAL CHEMISTRY (**1**, 424 [1955]):

> ". . . . beginning with January 1, 1958, those associate members who will have been at that time associate members for a period of 4 years shall be assessed full member dues. After that date *newly elected* associate members shall be permitted 4 years at the reduced dues. Assessment to full member dues shall not be construed as promotion to full membership."

Associate members will be billed for 1958 dues in accordance with the above regulation. Those associate members who believe they now meet full member requirements and desire a change in their status may communicate to that effect to Dr. Harry Sobotka, the Mount Sinai Hospital, New York 29, N. Y. An outline of requirements for full membership will be sent on request.

CLINICAL CHEMISTRY SESSIONS AT AAAC MEETING, INDIANAPOLIS

The Session of the American Association of Clinical Chemists will be held during the 124th Meeting of the American Association for the Advancement of Science, December 26-30, 1957, Indianapolis, Ind.

General Session 1, December 27, 1957, 9:00 a.m., Hotel Severin

Chairman: DONALD E. BOWMAN, Indiana University, School of Medicine, Department of Biochemistry, Indianapolis, Indiana

1. Can Dietary Protein Deficiency be Assessed Biochemically? S. C. WERCH, G. LEWIS, and J. H. FERGUSON, Departments of Obstetrics-Gynecology and Biochemistry, University of Miami School of Medicine, Miami, Fla.

2. Urinary Calculi: I. A Simple Semi-Quantitative Method of Analysis. HENRY O. NICHOLAS, Department of Chemistry, The Rice Institute, Houston, Texas.

3. Urinary Calculi: II. Classification of Calculi Found in the Southeast Texas Area. HENRY O. NICHOLAS

and H. F. LEIFESTE, Department of Chemistry, The Rice Institute and the Hermann Hospital, Houston, Texas.

4. Studies of Peroxidase and Occult Blood in Stool. ERNEST C. ADAMS, JR., ALFRED H. FREE, and GALEN F. COLLINS, Miles-Ames Research Laboratory and Miles-Ames Pharmaceutical Research Laboratory, Elkhart, Indiana.

5. The Extraction and Chromatography of Organic Acids from Body Fluids. S. MEITES, Children's Hospital, Columbus, Ohio.

6. A System of Clinical Chemical Analysis. BERNARD KLEIN, Biochemistry Laboratory, Veterans Administration Hospital, Bronx, N. Y.

7. Some Possible Applications of Redox Potential Measurements in Clinical Laboratories. H. E. WILCOX, Department of Chemistry, Birmingham Southern College, Birmingham, Ala.

8. Toxicity of Boron Hydrides. JOSEPH L. SVIRBELY, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

General Session 2, December 28, 1957, 2:00 p.m., Warren Hotel

Chairman: OLIVER H. GAEBLER, Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit 2, Mich.

1. Blood pH Determination: The Validity of Temperature Correction Factors. WILLARD R. FAULKNER, Cleveland Clinic Foundation, Cleveland 6, Ohio.

2. Studies on the Determination of Hemoglobin as Cyanmethemoglobin, BESS G. OSGOOD, Department of Chemistry, South Bend Medical Foundation, Inc., South Bend 1, Ind.

3. Determination of Serum Copper and Iron. BENNIE ZAK, WILLIAM WALTERS, and JAMES LANDERS, Laboratories of the Department of Pathology, Wayne State University College of Medicine, and Detroit Receiving Hospital.

4. An Improved Method for Determining Urinary Catecholamines. ROY B. JOHNSON, JR., Division of Laboratories, Scripps Clinic and Research Foundation, La Jolla, Calif.

5. The Use of a Strip of Rabbit Aorta for Estimating the Catecholamine Content of the Urine as an Aid in the Diagnosis of Pheochromocytoma, O. M. HELMER, Lilly Laboratory for Clinical Research, Indianapolis General Hospital, Indianapolis, Ind.

6. Correlation of Ascorbic Acid Content and Lag Period in Oxidation of *N,N*-Dimethyl-*p*-phenylenediamine by Sera From Normal and Psychotic Humans. M. H. APRISON and F. J. GROSS, Institute of Psychiatric Research, Department of Psychiatry, Indiana University Medical Center.

7. The Determination of the Daily Production of Hydrocortisone in Man by Means of Hydrocortisone-4-C^{14}. HAROLD PERSKY, Institute of Psychiatric Research, Department of Psychiatry, Indiana University Medical Center.

8. Nitroprusside Reactive Compounds in Blood and Urine in Ketosis, ROBERT R. SMEBY, HELEN M. FREE, and MARION H. COOK, Miles-Ames Research Laboratory, Elkhart, Ind.

Symposium: "Significant Trends in the Chemistry of Disease," December 28, 1957, 9:00 a.m., Hotel Severin

Chairman: ALFRED H. FREE, Miles-Ames Research Laboratory, Elkhart, Ind.

1. Electrolytes and Acid-Base Balance. HARRY WEISBERG, The Chicago Medical School and the Little Company of Mary Hospital, Chicago, Ill.

2. Chemical Changes Involved in the Use of Artificial Organs. JACK R. LEONARDS, Western Reserve University Medical School, Cleveland, Ohio.

3. Recent Advances in the Understanding of Hormonal Factors in Disease. RALPH I. DORFMAN, Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

4. Use of Serum Transaminase Activities in Clinical Biochemistry. CLARENCE COHN, Department of Biochemistry, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

Dinner, Clinical Chemists. Friday evening December 27 at 7.00 P.M. at the Hotel Severin.

ERNST BISCHOFF AWARD TO JOSEPH H. GAST

The sixth Ernst Bischoff Award was presented at the annual dinner of the Association in New York City on September 12, 1957. The recipient was Joseph H. Gast, Professor of Biochemistry at the Baylor University College of Medicine.

Dr. Gast received his education at the University of Michigan where he was awarded a doctorate degree. He has been affiliated with Baylor University since 1942.

The ceremonies were presided over by Dr. Joseph I. Routh, President of the AACC, and Dr. Gast was cited "For his efforts in advancing the practice of Clinical Chemistry on behalf of the patient, the physician, and the chemist; for his insistence on intelligence and honesty in selection, execution, and interpretation of chemical determinations; for his contributions to the broad scientific basis on which Clinical Chemistry rests; and for his endeavors in the professional attainments of the clinical chemist."

In his address Dr. Gast discussed the development of a field of "chemical electronics" for the measurements of the state of body functions and pointed out the limitations of the present single function analyses. The thought-provoking address was received with a great deal of interest.

The Ernst Bischoff Award is sponsored by the Ames Company of Elkhart, Indiana, and is given annually by the AACC to a scientist for outstanding contributions in clinical chemistry. The Award consists of a scroll, a medal, and an honorarium of $500.00. The recipient is selected by an Award Committee of the Association.

Procedure for nominations for the Ernst Bischoff Award has been described in CLINICAL CHEMISTRY [2, 449 (1956)]. Nominations for the 1958 Award should be received by the National Secretary not later than April 15, 1958. It is hoped that the matter of nominations will be placed

on the agenda of all local sections and individual members likewise will participate.

TENTH ANNUAL MEETINGS

The tenth annual meetings of the American Association of Clinical Chemists will depart in format from the meetings of preceding years. The scientific sessions will be held on the campus of the State University of Iowa in Iowa City. September 4-6, 1958, has been chosen as the date for these meetings so that interested members may attend the American Chemical Society meetings which will be held during the following week in Chicago. Plans for the program are currently being formulated, and it is anticipated that the presentations will include submitted papers and symposia during the 2½-day sessions. Committees are being chosen to assist in organizational preliminaries. Further details will be announced in the near future.

Iowa City is served by both the Rock Island Railroad and United Airlines. Housing and meals will be available in the university dormitories at very modest rates. We hope the membership and other interested individuals will reserve these dates and plan to attend the meetings.

REPORTS FROM THE SECTIONS

Philadelphia

The Philadelphia Section of the American Association of Clinical Chemists at its Forty-sixth Scientific Meeting heard papers on technics for measurement of serum potassium and urinary iron.

Dr. F. William Sunderman, Jr., Fellow in Metabolic Research at Jefferson Hospital, spoke on the turbidimetric tetraphenylboron technic for measurement of serum potassium. Sources of reagent instability, nonlinear results, and positive errors in measurement were noted, and remedies suggested. The effects of *p*H, time, temperature, and reagent concentration were discussed. Ethylenediaminetetraacetate was found to eliminate a source of error, which was shown not to be calcium or magnesium. Formaldehyde was used to eliminate a source of error assumed to be from amine compounds. The new technic showed good correlation with results from flame photometric and platinic chloride methods for potassium.

Dr. Marvin J. Seven, now completing a medical residency at Philadelphia General Hospital, and formerly at the National Institutes of Health, described a technic for the determination of iron in urine. A description was given of a fume duct adapted to Erlenmeyer flasks, which simplified digestion procedures. Precautions were noted in technics and reagents for adequate digestion of samples. Reduction was accomplished by use of hydroxylamine hydrochloride, and color developed as the bathophenanthroline complex. Operating conditions eliminated interferences from cobalt and copper. Recovery studies with radioactive iron showed color enhancement with the use of appropriate acetate buffers.

Both of the above papers have been submitted for publication.

A brief report on the recent International Congress of Clinical Chemistry at Stockholm was given by Miss Margaret E. Ryland of the Woman's Medical College of Pennsylvania.

The meeting was held in the new Nurses Residence at the Presbyterian Hospital in Philadelphia on October 8, 1957.

PEACE PAUBIONSKY

Metropolitan New York

A Nominating Committee for the Van Slyke Award in Clinical Chemistry has been approved by the Executive Committee of the New York Section. The committee consists of Dr. Israel Kleiner of the New York Medical College as chairman, Dr. Reginald Archibald of the Rockefeller Institute and Dr. Abraham White of the Albert Einstein College of Medicine. This committee has consented to select the future recipients of the Van Slyke Award. The next presentation of this Award is planned for the meeting of the New York Section early in January, 1958. The choice of the candidate for this Award is restricted to persons working or having performed their major work in the metropolitan New York area and is given for outstanding work in the field of clinical chemistry in the tradition established by Dr. Donald D. Van Slyke.

A. SAIFER

Boston

The following have been elected as officers of the Boston Section of the coming year: *Chairman:* Moira D. Reynolds, Boston University Medical School. *Vice-Chairman:* Esther Thomas, Boston, Mass. *Secretary.* Joseph Benotti, Boston Medical Laboratory, 19 Bay State Road, Boston, Mass.

Texas

The Texas Section has met in conjunction with the Fall Meeting of the Southwestern Section of the Society for Experimental Biology and Medicine, at Texas A & M College, College Station, on November 1 and 2, 1957. The annual meeting was held at a luncheon on Saturday, November 2. This was the last meeting of 1957.

RUSSEL O. BOWMAN

CLEVELAND SYMPOSIUM ON CLINICAL CHEMISTRY METHODS

Following is a list of the papers presented at the Symposium on Clinical Chemistry Methods, held at The Frank E. Bunts Educational Institute, Cleveland, Ohio, November 13-15, 1957.

LIVER FUNCTION: I

Chairman: Adrian Hainline, Jr.

Chemical Disturbances Associated with Liver Disease, JOHN G. REINHOLD

The Clinical Significance in Alterations in Serum Transaminase, FELIX WROBLEWSKI

Factors Which Influence the Turbidity and Flocculation Tests, JOHN G. REINHOLD

LIVER FUNCTION: II

Chairman: Roger W. Marsters

Procedures and Pitfalls in Porphyrin Analysis, SAMUEL SCHWARTZ

The Quantitative Measurement of Bile Pigment, J. WAIDE PRICE

Electrophoresis of Bile, MIRIAM REINER

The Determination of Tissue Glycogen, ADRIAN HAINLINE, Jr.

The Application of Chemical Measurements to the Study of the Liver and Biliary Tract, JOHN G. REINHOLD

Horizons in Porphyrin Research, SAMUEL SCHWARTZ

ELECTROPHORESIS: I

Chairman: Lena A. Lewis

Application of Electrophoresis in the Clinical Laboratory, MIRIAM REINER

Practical Aspects of Paper Electrophoresis, WILLARD R. FAULKNER

Determination of Total Protein, JOHN G. REINHOLD

Normal Values in Electrophoresis, LENA A. LEWIS

ELECTROPHORESIS: II

Chairman: Miriam Reiner

Electrophoretic Analysis of Fluids other than Serum, LENA A. LEWIS

Application of Electrophoresis to Miscellaneous Media, MIRIAM REINER

Electrophoretic Determination of Hemoglobin, LENA A. LEWIS

MICROCHEMISTRY

Chairman: Adrian Hainline, Jr.

Basic Principles of Micro Techniques As Applied to the Clinical Laboratory and their Application to Diagnosis, SAMUEL NATELSON

Experiences in the Development of Micro Techniques, WILLARD R. FAULKNER

Microdiffusion, WILLARD R. FAULKNER

Micro Techniques and their Application to Fluid and Electrolyte Balance, SAMUEL NATELSON

DETERMINATION OF ENZYMATIC ACTIVITY

Chairman: J. Waide Price

Basic Factors in Measurements of Enzyme Activities, FELIX WROBLEWSKI

The Amylase Determination, ROGER W. MARSTERS

The Determination of Phosphatase, JOHN W. KING

The Clinical Significance of Alteration of Lactic Acid Dehydrogenase Activity in Body Fluids, FELIX WROBLEWSKI

In addition to scheduled papers and panel discussions, the meeting included a "mixer" at Fenway Hall and several tours of chemistry laboratories in the area.

Erratum

In the paper by E. J. King in the Supplement to the August, 1957, issue of CLINICAL CHEMISTRY (Vol. 3, No. 4, Part Two), pages 510 and 511 do not appear. This is due to an error in pagination only, and the text is complete and in proper order. In the same paper, the insert consisting of Figs. 1 and 5, which should have appeared between pages 508 and 509, was incorrectly placed and will be found between pages 546 and 547.

BOOK REVIEWS

Biochemical Disorders in Human Disease. R. H. S. THOMPSON and E. J. KING (Eds.). *New York, Academic Press, Inc.*, 1957, 843 pp., $12.60

The importance of biochemistry in the world of medicine has grown to a tremendous extent in the past quarter-century. The present-day physician, whether internist, pathologist, or surgeon, has frequent need to turn to the biochemist for help in the resolution of his problems. A book to serve as a source of information in "morbid biochemistry" comparable to the standard texts in morbid anatomy had not been available. The impact which biochemistry has had on virtually every aspect of human disease makes the problem of incorporating all such information between the covers of one book a most formidable one. Professors Thompson and King deserve great credit for the success they have had with this volume in achieving a solution to this problem.

The editors, both of whom are experienced teachers of chemical pathology, have enlisted as authors a distinguished company of British and American teachers and investigators which includes themselves. The book consists of twenty separate monographs and incorporates an enormous fund of information. The facts as presented and their interpretation will prove of great assistance to any physician who has occasion to refer to any of the subjects treated here. The different sections vary a great deal in method of organization and also in quality of material and thoroughness. The unevenness of subject presentation which almost inevitably seems to result when many writers are engaged is, unhappily, present here as well. This fact will probably not constitute too much of a handicap inasmuch as the book is intended for reference use. It would serve no useful purpose to single out individual authors for praise or censure. The editors are responsible for the organization of the work and also for the inclusion or omission of subject matter, so it is to them that the criticism is directed.

It is stated that the book was organized on an organ basis rather than in the classic manner of texts on biochemistry, which usually discuss metabolism of proteins, fats, carbohydrates, pigments, etc. However, they have included six chapters on "Miscellaneous Disorders of Metabolism," while discussion of diseases of the heart and lungs is conspicuously absent. There is no mention of any of the recent important studies on oxygen and carbon dioxide tension in the blood in cardiopulmonary disease. The sections on diseases of the blood omit any mention of diseases of white blood cells. There are no reports on enzyme studies in leukemias, leukoses, or leukemoid reactions. Multiple myeloma is mentioned briefly twice, but there is no information on the serum proteins in this disease. Paper electrophoresis of serum proteins receives scant

mention in the chapter "Diseases of the Kidney and Genito-Urinary Tract," but is not dealt with in detail anywhere in the book. The propriety of having some of the authors write sections on therapy is probably a matter of personal preference. It is difficult to reconcile such material in a book on chemical pathology.

Some of the omissions may be a result of publishers' deadlines, but it does seem strange to find no reference to serum transaminases in the section on "Diseases of the Liver and Biliary Tract" in a book published as late as July, 1957. The section on pancreas contains nothing on the biochemical disorders associated with fibrocystic disease of the pancreas. This lack becomes even more deplorable when one realizes that Dr. Dorothy Andersen is the author of the section on "Glycogen Storage Diseases and Galactosaemia." The editors plead space limitations to excuse the omission of a section on cancer, but the great importance of this problem in medicine is eloquent plea for its inclusion in a work of this type.

These criticisms are offered because the book is a good one and will form a valuable addition to any medical library. I feel that future editions may overcome these omissions and make the book even more useful. Certainly one has every right to expect a truly comprehensive text after contemplating the rich talent engaged in its production.

The editors state that they hope that the book may prove of value to their colleagues in biochemistry to put problems in research before them and to help them to understand the needs of physicians. In this respect, it would seem that their hopes are vain ones. Certainly the informed clinical chemist would be in most instances in possession of the information as presented in the text. The book should enable him to give to his medical colleagues a reference work to which they can make frequent inquiry.

In summary, Professors Thompson and King have made available to British and American physicians a book which provides them with an informed picture of the biochemical disorders involved in certain diseases. The text is clear and concise and reads well. It incorporates a very comprehensive bibliography in each subject to enable the reader to pursue the subject in greater detail if such be needed. This is a book which should take its place among the reference works in the library of every informed physician.

Lebanon Hospital IRVING M. RATNER
New York, N. Y.

A Practical Manual Of Medical and Biological Staining Techniques (ed. 2). E. GURR. *New York, Interscience Publishers,* 1957, $6.50

While this manual in its first edition established itself as a very valuable guide in staining technics, its second edition has widened the scope in this field by the addition of methods of more recent development. Yet there is

still left open a possible widening of this ever-increasing subject in a third edition.

The clear-cut tabulation of substances, procedures, and their respective fields of application have proved a most valuable feature. This tabulation, supported by brevity of language, greatly facilitates the otherwise complex and time-consuming literature searches for specific histologic procedures, whether applied to medicine or other sciences.

The new section on histochemical methods constitutes a very important addition, putting in a comprehensive form the procedures of this less frequently applied method of investigation.

Metropolitan Hospital, New York, N. Y. GRETA STOHR

An Introduction to Blood Group Serology. K. BOORMAN and B. E. DODD. (*Boston, Little, Brown & Co., 317 pp., 1957, $7.50*)

This new book by Boorman and Dodd is another addition to the literature on blood groups by British workers. The scope of the book can be seen from the chapter headings, which include the A-B-O blood group system, the Rh blood group system, the M-N-S system, and other blood group systems; practical application of blood group theory in blood transfusion; hemolytic disease of the newborn; hemolytic anemia; disputed paternity; anthropology; and a chapter on apparatus and reagents. The usefulness of the volume is enhanced by an appendix containing practical pointers for the technician, a glossary, and an index of technics.

Kathleen Boorman and Barbara Dodd are two of the leading blood-group workers in England, and this volume which represents the fruits of their own rich practical experience is a useful guide to the subject, as well as a simple introduction to the field. This is all the more remarkable since these authors are saddled with a handicap which they share with other British workers, namely, they are committed to a serologic concept, a genetic theory, and nomenclature which do not correspond with the observable facts. The genetic theory and nomenclature were suggested by the famous British mathematician R. A. Fisher, in 1944. While Fisher is one of the world's leading biometricians, he has never done any blood-grouping work and is unfamiliar with its vast literature, so he was unaware that the same linkage theory had been disproved in 1942 by Wiener, who instead proposed a theory of multiple allelic genes. Nevertheless, so great is Fisher's prestige, due to his pathfinding mathematical discoveries of 30 years ago, that workers, especially in England, have blindly followed his lead even when the results of their own research on the Rh-Hr types contradict the Fisher concept. In fact, certain chauvinistic workers go so far as to distort and misrepresent facts in order to reconcile them with Fisher's preconceived ideas. An outstanding example is the citation of the supposed discovery of little d-serum as proof of Fisher's concept, and the

fact that these reports could not be confirmed has not deterred Boorman and Dodd from repeating in their book Fisher's simple but incorrect scheme of 6 Rh antiserums and corresponding antigens, namely, anti-C, anti-D, anti-E, anti-c, anti-d, and anti-e (cf. p. 112). This oversimplified concept is unrealistic and evades the complexities of the subject, such as those resulting from the discovery of factors **rh**^{w} (C^{w}), **hr** (f), and the recently reported factors **Rh**^{A}, **Rh**^{B}, **Rh**^{C}, etc. In common with other C-D-E- protagonists, moreover, Boorman and Dodd fail to distinguish sharply between phenotypes and genotypes, which is blameworthy as they themselves admit (p. 107), and some of the confusing consequences of this practice can be found in tables 19 and 20 on pages 92 and 95.

In a recent report of the Committee on Medicolegal Problems of the American Medical Association, which appeared in the Journal of the American Medical Association for August 31, 1957, it is recommended that in medicolegal reports the standard Rh-Hr nomenclature be used exclusively and the C-D-E notations be avoided. The time has arrived, when for the sake of scientific accuracy and to avoid confusion, this recommendation must be extended also to the application of the Rh-Hr typing in clinical medicine and anthropology. There is no room in this review to repeat the great mass of scientific evidence on which the recommendation of the A.M.A. report is based. Therefore, it is urged that anyone who wishes to use the Boorman-Dodd book should first study the A.M.A. report and read the references cited therein. Only in this way is it possible to prevent the perpetuation of the misinformation and lack of the full knowledge of the subject on which the continued use of the C-D-E notations depends.

In view of these facts, the book of Boorman and Dodd is recommended, but only to those readers who have first taken the trouble to familiarize themselves with the difference between a blood factor and an agglutinogen, a concept which is just as fundamental to blood group serology as Newton's laws of motion are to mechanics. Those who fail to study all the available evidence may be trapped into misconceptions and misunderstanding by a nomenclature which does not faithfully reflect the laboratory observations.

Division of Immunohematology A. S. Wiener
The Jewish Hospital of Brooklyn (N. Y.)

Paper Electrophoresis. G. E. W. Wolstenholme and Elaine C. P. Millar (Eds.). (*Boston, Mass., published by Little, Brown and Co. for the Ciba Foundation,* 1956, 224 pp., $6.50)

This book is not meant for the use of beginners in the field but, as is customary in the Ciba Symposia, consists of a series of papers by a group of established experts in paper electrophoresis. As such, there is a good deal of discussion on important theoretical aspects of protein separation by paper electro-

phoresis by authorities such as Harry Svensson in his chapter "Physicochemical Aspects and their Relationship to the Design of Apparatus," by J. De Wael in "The Combined Influence of Evaporation and Diffusion on the Separation of Serum Proteins by Paper Electrophoresis," and by Goeffrey Franglen in "Protein-Dye Interactions Considered in Relation to the Estimation of Protein in Paper Electrophoresis." There is also throughout the book a great deal of informative material on the important problems of the binding of ions to proteins and the specific effect of different kinds of buffers on the type of electrophoretic pattern obtained.

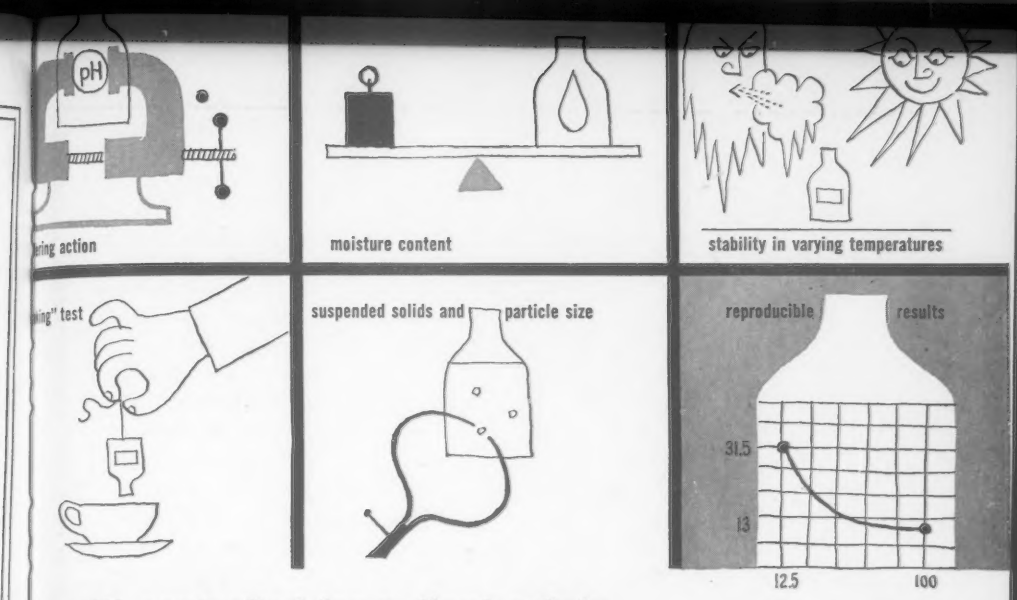
There are, however, a number of chapters that will give the reader interested in establishing electrophoretic technics in his laboratory a great deal of useful information. These include "General Methods of Paper Electrophoresis with Examples of Its Use in Medical and Biochemical Problems" by W. Grassmann; an excellent article on the "Analysis of Human Haemoglobins by Paper Electrophoresis" by J. C. White, G. H. Beaven, and M. Ellis; and an article by Hugh J. McDonald on "A New Approach to the Staining of Lipoproteins" and articles by N. H. Martin and E. M. Crook on the "Analysis of Separated Materials." Perhaps most important of all from the point of view of future development of the field of paper electrophoresis are articles on "High Voltage Paper Electrophoresis" by B. Kickhofen and a general philosophical discussion by one of the American founders of this field, E. L. Durrum, on "The Future of the Technique in Its Application to Clinical Research and Routine Analysis."

In summary, the book contains much useful information for investigators already experienced in this technic. The only article which would be of great interest to beginners interested in applying paper electrophoresis to the study of hematological diseases is that dealing with human haemoglobins by J. C. White *et al.*

Isaac Albert Research Institute A. SAIFER
Brooklyn 3, N. Y.

Index to Volume 3

Index to Advertisers



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